GYPSY MOTH EGG DEVELOPMENT - A MODEL OF PHENOLOGICAL EVENTS

by
David Richard Gray

Dissertation submitted to the Faculty of the
Virginia Polytechnic Insitiute and State University
in partial fulfillment of the requirment for the degree of
DOCTOR OF PHILOSOPHY
in
Entomology

APPROVED:

F. W. Ravlin
Chairman

J. A. Logan

D. E. Mullins

M. L. McManus

N. D. Stone

January, 1994
Blacksburg, VA
24061-0319

GYPSY MOTH EGG DEVELOPMENT - A MODEL OF PHENOLOGICAL EVENTS

ABSTRACT

A phenological model of gypsy moth egg development is proposed that distinguishes three phases of egg development, prediapause, diapause and postdiapause. A technique of measuring respiration rates of individual eggs was developed and respiration rate was used as a physiological variable to distinguish the phases. The pattern of respiration rate provided strong evidence in support of three distinct developmental phases. Respiration rate developed embryos declined sharply as prediapause was entered and rose sharply when diapause was completed. When the effect of age on respiration rate was removed, temperature had a uniform effect on respiration rate throughout the egg stage. A 10°C decrease in temperature caused an approximate 0.4 fold decrease in respiration rate, indicating that eggs in diapause are as equally responsive to temperature as egg in a nondiapause phase.

Developmental rate in prediapause was strongly temperature-dependent, and the relationship was described by a non-linear function. Prediapause duration was approximately 13 days at 31°C. The depletion of stored triglycerides was strongly linked to the completion of prediapause

Developmental rate in postdiapause was found to be temperature- and age-dependent. Developmental response to temperature was relatively weak and linear at the onset of postdiapause. As postdiapause advanced, the response became stronger and non-linear. The temperature- and age-dependent developmental response was fully described by the temperature-dependent developmental response at the onset of postdiapause, and by a temperature-dependent rate change parameter.

ACKNOWLEDGMENTS

I am deeply indebted to my major advisor, Dr. William Ravlin. He did much more than just provide the opportunity to do research. He provided a stimulating environment in which to do it. He provided advice and guidance that went beyond the field of science. He provided moral and emotional support that went far beyond any responsibilities as an advisor. And he offered a friendship that I value very highly. More than any other person, he is responsible for making my time at V.P.I. & S.U. a rewarding, and truly enjoyable experience.

I am also deeply indebted to Dr. Jacques Régnière for the selfless sharing of his time and very considerable talents during his one year sabbatical at V.P.I. & S.U. His contributions to my research efforts, and to my understanding of phenological modeling were enormous.

I would like to thank the rest of my committee, Dr. Jesse Logan, Dr. Michael McManus, Dr. Nicholas Stone and Dr. Donald Mullins whose doors were always open. A special thanks is due Dr. Logan for his considerable contribution to my education.

Everybody thanks their "significant other", but mine deserves it more than any I can imagine. She encouraged me when I needed it, and assisted me in so many aspects of my work. Dr. Jodi Carlson has been a challenging partner with whom I have been able to share countless hours of fruitful scientific discussion. She has contributed hugely to the quality of my life, and in 7 days I will be honored to be her husband.

Many other people deserve thanks for their generous help. I thank Dr. Robert Bell for many helpful discussions on the physiology of gypsy moth eggs and for providing much of my research material. I thank Dr. Shelby Fleischer for the many helpful discussions on experimental design we had early in my career at V.P.I. & S.U. I thank Mr. Frank Rock for his carpentry skills and for his very high standards of workmanship. And I thank fellow students David Judge and Keith Tignor for their invaluable assistance in the lab.

And lastly, I wish to thank Mr. Andy Roberts. He provided help at work, diversion away from it, and a cherished friendship in both.

TABLE OF CONTENTS

List of Tables

List of Figures

Chapter 1

Introduction

Benefits of a phenological model of gypsy moth egg development. There has been extensive research on the effects of temperature on gypsy moth egg development and several phenological models have been developed (Johnson et al. 1983, Waggoner 1984, Lyons and Lysyk 1988, Sawyer et al. 1993). However, the precise nature in which gypsy moth eggs respond to temperature throughout the developmental stage remains uncertain.

An interest exists in the relationship between temperature and developmental response in gypsy moth eggs for several reasons. A basic necessity of insects living in seasonal climates is the maintenance of an appropriate relationship between life cycle and season. For example, egg hatch and larval feeding must coincide with the occurrence of adequate food. 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, which encompasses a diapause, is a critical component of its phenology.

Phenological studies have additional importance when the species involved is of economic importance (Logan et al. 1990). Such is the case with gypsy moth egg phenology. There is a trend toward the use of microbial insecticides that may be less harmful to the environment than previously commonly used insecticides. The efficacy of microbial agents such as *Bacillus thuringiensis* (Bt) and nucleopolyhedrosis virus (NPV) is highly dependent upon application of the agent with the highly susceptible second instar larvae. Prediction of the time of occurrence of this susceptible stage can be difficult when the prediction must be made over a large geographic area with significant temperature differences. Phenological models of larval development (Casagrande et al.

1987, Logan et al. 1990) have been developed and extended to a landscape scale (Schaub et al. 1994) to aid in this prediction. However model predictions are dependent on the date of egg hatch which may be unknown. Predictions of egg hatch on a landscape scale would be a valuable addition.

A newly emerging tactic for suppression of gypsy moth populations involves the dispersion of egg masses from "substerilized" gypsy moth adults. Larvae from the egg masses mature into sterile adults. Subsequent matings between sterile and wild adults result in unembryonated eggs. Developmental rates of the sterile larvae are similar to those of the wild larvae. Therefore, coincident hatch of the sterile and wild populations is important to the probability of mating between the two. Prediction of egg hatch of the wild population is essential. In addition, a full understanding of the effects of temperature on egg development is essential if laboratory conditions are to be manipulated to result in appropriate development of the sterile population.

Since its accidental release in Boston Massachusetts in 1868 or 1869 by Etienne Leopold Truvelot (Liebhold 1989) the gypsy moth has expanded its range from southern Quebec and Ontario to South Carolina in the east and Michigan in the west. Populations have been detected in Missouri and several western state including Washington, Oregon, California and Utah. Gypsy moth larvae feed on over 300 species of trees and shrubs (Lechowicz and Mauffette 1986) and with its broad range of suitable host material the gypsy moth may eventually occupy most of North America. Guidelines of the Animal and Plant Health Inspection Service for detection of gypsy moth populations rely only on the abundance of potential hosts and the risk of egg mass importation as criteria for determining the extent of detection efforts (Foltz, pers. comm.). However, the range of the gypsy moth may ultimately be limited not by available host material, but by temperatures insufficiently cold for diapause completion in the south (Allen et al. 1993),

or insufficiently warm for prediapause completion in the north (Gray et al. 1993). A fuller understanding of the effects of temperature on gypsy moth egg development would be useful when deploying limited resources in detection activities.

Gypsy moth egg development. The gypsy moth is a univoltine species with an obligatory diapause period and spends about 9 months in the eggs stage as a rather mature larva (Bell 1989). The ontogeny of gypsy moth eggs is commonly described as being comprised of three phases. Eggs are laid in mid- to late-summer. The initial phase of prediapause is characterized by morphological development of the embryo. Bell (1989) stated that embryos appear as fully differentiated larvae, but not fully competent, a few days before the onset of diapause. During the diapause phase morphological development is arrested and eggs are in a physiological state that enhances their ability to survive winter conditions. During postdiapause eggs become fully competent and hatch in the spring.

Temperature is the most important variable influencing gypsy moth egg development. Many authors have reported significant effects of temperature on egg development and hatch (Masaki 1956, Pantyukhov 1962, Tauber et al. 1990, Sanderson and Peairs, 1913). Unlike many insects, gypsy moth egg development does not appear to be strongly influenced by photoperiod. Danilevski (1956) recorded gypsy moth within a list of eight species of Lepidoptera that are photoperiod neutral. Leonard (1968) confirmed the absence of a developmental response to photoperiod. In contrast, Tauber et al. (1990) concluded that short days decrease the rate of diapause development if they occur before eggs experience cold temperatures. After exposure to cold temperatures photoperiod has no effect.

Surprisingly little is known of the physiology of gypsy moth eggs (Bell 1989) or of the physiology of late embryonic diapause of any insect species (Denlinger 1984). Bell

(1989) reported that the brain and central nervous system of developing gypsy moth embryos were differentiated after 7 -8 days of incubation at 25°C. Formation and deposition of the embryonic larval cuticle occurred by day 12 - 14, and the embryo appeared fully differentiated by day 14 - 15. By day 19 - 20 metabolic rate began to decline, indicating the onset of diapause. Nothing is known of the endocrine role in diapause induction, although Bell (1989) suggests that juvenile hormone may play a role.

Gypsy moth eggs are freezing-intolerant (as per Lee 1991) and eggs are capable of withstanding sub-freezing temperatures very early after the onset of diapause, or sooner. Pantyukhov (1964) reported supercooling points of approx. -20°C in mid-October without prior exposure to cold temperatures. It is not known how earlier supercooling points compared to this. The specific cryoprotectants used by gypsy moth eggs have not been identified, but glycerol and other polyhydric alcohols are probably responsible, as in many other insect species (Storey and Storey 1991). Madrid (1979) reported an increase in glycerol and glycoprotein levels during winter. A likely factor in the common use of glycerol as a cryoprotectant is the presence of constitutive pathways for its synthesis and catabolism in the insect fat body as part of lipid metabolism (Storey and Storey 1991).

The relationship between diapause and cold hardiness is unclear (Denlinger 1991). It has been argued that diapause and cold hardiness are independent (Ring 1972) and dependent (Mansingh 1974) events. But it is likely that as either a component of the process preparing a gypsy moth egg for diapause initiation, or as an independent but simultaneous event, the necessary conditions for surviving sub-freezing temperatures are being prepared during prediapause as in the eastern tent caterpillar (Mansingh 1974). However, supercooling point may be further lowered by the accumulation of additional glycerol that accompanies exposure to cold temperatures (Mansingh 1974). Glycerol is

likely converted to glucose and perhaps to lipids at the termination of diapause (Mansingh and Smallman 1972).

Lipids may be the principle energy source during gypsy moth diapause. Chino (1958) suggested that lipids are the major metabolic energy source during diapause of *B. mori*. Yamashita and Hesegawa (1985) stated that Nakasone (1979) found that triglycerides accounted for 60% of the total CO_2 expired, while phospholipid and sterol levels remained unchanged during *B. mori* embryogenesis. Chippendale (1973) found lipids formed the principal metabolic reserve of diapausing southwestern corn borer, a species with an homologous diapause (Bell 1989). Adedokun and Denlinger (1985) reported that lipids were the principal metabolic reserve of diapausing flesh flies and that a transition to carbohydrate utilization in mid-diapause may signal an end to a period of "fixed latency ".

Phenological models of gypsy moth eggs. In conventional experiments investigating the developmental rates to be used in a phenological model, 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. 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.

Difficulties associated with determining the effect of temperature on egg developmental rate and then developing a phenological model may be largely due to the difficulty in distinguishing the phases of egg development (Lyons and Lysyk 1988) and the concomitant difficulty in quantitatively describing the possibly unique developmental response to temperature exhibited by each phase. Some phenological models of egg

development avoid distinguishing the phases and assume that diapause is completed by a largely arbitrarily chosen date. They assume that the developmental response to temperature is uniform in postdiapause and model developmental rate as linearly (Johnson et al. 1983) or non-linearly (Waggoner 1984, Lyons and Lysyk 1989) dependent on temperature. Therefore, these models are more accurately classified as phenological models of egg hatch, and not of egg development. These models were fit to egg hatch data from specific areas in North America, and the assumption of date of diapause completion can lead to gross errors in simulated egg hatch dates (up to 25 days, Smith, pers. comm.) 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 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.

Research objectives. The goal of the research described in the following pages was to advance the understanding of the effects of temperature on the ontogeny of gypsy moth eggs. This goal was achieved through the studies presented in the following chapters. In addition, the design, construction and testing of a set of six environmental chambers used in the experiments is described. The specific objectives of each study were as follows.

Chapter 2. Micro-processor controlled mini-environmental chambers capable of -10° to 30°C. The objective of this work was to design, construct, and test a set of six environmental chambers suitable for investigating the effects of temperature on the development of gypsy moth eggs.

Chapter 3. Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature-time requirements of prediapause development. The objectives of this study were to (1) develop and test a non-destructive technique for distinguishing developmental phases in individual insect eggs; (2) describe the temperature-dependent prediapause developmental rate of gypsy moth eggs; (3) describe variability in prediapause developmental rate of a population of gypsy moth eggs; and (4) discuss the application of the technique to test current hypotheses regarding diapause termination in gypsy moth.

Chapter 4. A model of temperature-dependent depletion of stored triglyceride in gypsy moth eggs during prediapause development: implications for egg survival. The objectives of this study were to quantify the depletion of triglyceride

reserves in individual gypsy moth eggs and to develop a model of temperature-dependent triglyceride depletion during prediapause development.

Chapter 5. Further advances toward a model of gypsy moth egg phenology: thermal responsiveness during diapause and age-dependent developmental rates in postdiapause. The objectives of this study were to (1) examine the pattern of thermal responsiveness in diapausing and postdiapausing gypsy moth eggs; and (2) to analyze and model changes in the developmental rate response to temperature that occur during postdiapause.

Literature Cited

Adedokun, T. A., and D. L. Denlinger. 1985. Metabolic reserves associated with pupal diapause in the flesh fly, *Sarcophaga crassipalpis*. J. Insect Physiol. 31: 229-233.

Allen, J. C., J. L. Foltz, W. N. Dixon, A. M. Liebhold, J. M. Colbert, J. Regniere, D. R. Gray, J. Wilder, and I. Christie. 1993. Will the gypsy moth become a pest in Florida? Florida Entomologist 76 (1):102-113

Bell, R. A. 1989. Respiratory activity during embryonic development in a diapausing and a selected non-diapausing strain of the gypsy moth, *Lymantria dispar* L. Comp. Biochem. & Physiol. 93A: 761-771.

Casagrande, R. A., P. A. Logan, and W. E. Wallner. 1987. Phenological model for gypsy moth, Lymantria, dispar (L.) (Lepidoptera: Lymantriidae), larvae and pupae. Environ. Entomol. 16: 556-562.

Chino, H. 1958. Carbohydrate metabolism in the diapause egg of the silworm, *Bombyx mori* - II. Conversion of glycogen into sorbitol and glycerol during diapause. J. Insect Physiol. 2: 1-12.

Chippendale, G. M. 1973. Diapause of the southwestern corn borer, Diatraea grandiosella: utilization of fat body and haemolymph reserves. Entomol. Exp. Appl. 16:395-406.

Danilevski, A. S. 1956. Photoperiodism and seasonal development of insects. Oliver and Boyd, Edinburgh and London. 283 pp.

Denlinger, D. L. 1984. Hormonal control of diapause. *In* G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Vol 8. Pergamon Press, Oxford.

Denlinger, D. L. 1991. Relationship between cold hardiness and diapause. *In* R. E. Lee and D. L. Denlinger [eds.], Insects at low temperatures. Chapman and Hall, New York.

Gray, D. R. , J. Regniere, R. W. Ravlin, and J. A. Logan. 1993. A comparison of three egg hatch models in eastern North America. USDA For. Serv. Gen. Tech. Rep. NE-179. 127 pp.

Johnson, P. C., D. P. Mason, S. L. Radke and K. T. Tracewski. 1983. Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), egg eclosion: degree-day accumulation. Environ. Entomol. 12: 929-932.

Lechowicz, M. J. and Y. Mauffette. 1986. Host preference of the gypsy moth in eastern North America versus European forests. Revue d'Entomologie du Quebec 31: 43-51.

Lee, R. L. 1991 Principles of insect low temperature tolerance. *In* R. E. Lee and D. L. Denlinger [eds.], Insects at low temperatures. Chapman and Hall, New York.

Leonard, D. E. 1968. Diapause in the gypsy moth. J. Econ. Entomol. 61(3):596-598.

Liebhold, A., V. Mastro, and P. W. Schaefer. 1989. Learning from the legacy of Leopold Trouvelot. Bull. Entomol. Soc. Am. 35(2): 20-22.

Logan, J. A., R. A. Casagrande, and A. M. Liebhold. 1990. Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. Environ. Entomol. 20(6):1516-1525.

Lyons, D. B. and T. J. Lysyk. 1988. Development and phenology of eggs of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), in Ontario, pp. 351-65. *In* Wallner, W.E. and K.A. McManus [eds.], Lymantriidae: a comparison of features of new and old world tussock moths. USDA Gen. Tech. Rep. NE-123.

Madrid, F. J. 1979. Laboratory and field studies on *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) in Quebec. Ph.D. thesis, McGill University.

Mansingh, A. 1974. Studies on insect dormancy. II. Relationship of cold-hardiness to diapause and quiescence in the eastern tent caterpillar, *Malacosoma americanum* (Fab.), (Lasiocampidae: Lepidoptera). Can. J. Zool. 52: 629-637.

Mansingh, A. and B. N. Smallman. 1972. Variation in polyhydric alcohol in relation to diapause and cold-hardiness in the larvae of *Isia isabella*. J. Insect Physiol. 18:1565-1571.

Masaki, S. 1956. The effects of temperature on the termination of diapause in the egg of *Lymantria dispar* Linne. Jpn. J. Appl. Zool. 21: 148-157.

Nakasone S. 1979. Changes in lipid components during the embryonic development of the silkworm, *Bombyx mori*. J. Sericult. Sci. Japan 48: 526-532. (In Japanese with English summary).

Pantyukhov, G. A. 1962. The effect of postive temperatues on different geographic populations of the European gold tail (*Euproctis chrysorrhea* L.) and the gypsy moth (*Lymantria dispar* L. - Lepidoptera, Orgyidae). Entomolo. Rev. 41(2): 169-175.

Pantyukhov, G. A. 1964. The effect of low temperatures on different populations of the brown-tailed moth *Euproctis chrysorrhoea* (L.) and the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Orgyidae). Entomol. Rev. 43: 47-55.

Ring, R. A. 1972. Relationship between diapause and supercooling in the blowfly, *Lucilia sericata* (Mg.) (Diptera: Calliphoridae). Can. J. Zool. 50:1601-1605.

Sanderson, E. D. and L. M. Peairs. 1913. The relation of temperature to insect life. 1. The variations in velocity of development at different constant temperatures. New Hampshire Agric. Exp. Stat. Tech. Bull., Durham, NH, No. 7.

Sawyer. A. J., M. J. Tauber, C. A. Tauber and J. R. Ruberson. 1993. Gypsy moth (Lepidoptera: Lymantriidae) egg development: a simulation analysis of laboratory and field data. Ecological Modelling 66:121-155.

Schaub, L. P., F. W. Ravlin, D. R. Gray, and J. A. Logan. 1994. A landscape-wide model for predicting gyspy moth (Lepidoptera: Lymantriidae) phenology. Environ. Entomol. (submitted).

Storey, K. B. and J. M. Storey. 1991. Biochemistry of Cryoprotectants. In R. E. Lee and D. L. Denlinger [eds.], Insects at low temperatures. Chapman and Hall, New York.

Tauber, M. J., C. A. Tauber and S. Masaki. 1986. Seasonal adaptations of insects. Oxford Univ. Press, New York. 411 pp.

Tauber, M. J., C. A. Tauber, J. R. Ruberson, A. J. Tauber and L. P. Abrahamson. 1990. Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. Ann. Entomol. Soc. Am. 83(3): 494-503.

Waggoner, P. E. 1984. The hatching of gypsy moth eggs, a phenological model. Agric. Forest Meteorol. 33: 53-65.

Yamashita O and. Hasegawa K. 1985. Embryonic diapause. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology* (Edited by Kerkut G. A. and Gilbert L. I.). Vol. 1, pp 408-434. Pergamon Press, Oxford.

Chapter 2

Micro-processor controlled mini-environmental chambers capable of -10° to 30° C.

Studies of the effects of temperature on insect development typically require several constant temperature regimes. This is particularly true when temperature responses are anticipated to be non-linear over the temperature range of interest. When the entire life-cycle of a temperate insect is under investigation, this range may extend from -10 to 35°C. In addition, reported differences (Roltsch et al. 1990, Tanigoshi et al. 1975) between developmental responses to fluctuating temperature versus a constant temperature equal to the mean of the fluctuating temperatures requires that some investigations include treatments of time-varying temperatures. Many available environmental chambers (ECs) are incapable of maintaining temperatures at or below ambient temperatures or of producing time-varying temperature regimes. The cost of a single low-temperature EC with photoperiod and time-varying temperature capabilities can severely limit the number of treatments in an experiment.

Several researchers have encountered this problem and their solutions have resulted in systems of varying natures. The EC described by Nicholls and Grills (1968) is capable of constant temperature and humidity regimes above ambient only. The microprocessor controlled ECs of Taylor and Shields (1990) produce temperatures very close to the program-requested constant or time-varying regime. In their system a single data acquisition and control system (DACS), in conjunction with a computer-resident control program written in Microsoft quickBASIC, monitors and controls temperature and photoperiod in eight independent chambers. A maximum of 10 daily, time-varying regimes is possible. The system relies upon a commercial low-temperature EC to heat and cool each chamber, limiting minimum temperatures to 0°C and resulting in a total cost of over $33,000, excluding the computer. The "Florida Reach-Ins" (Walker et al.

1992) are the most sophisticated of those described here. Temperature, relative humidity, and photoperiod are monitored and independently controlled in each of eight chambers by a single IBM XT computer running a 37,000 line (source code) Turbo Pascal program. Temperatures and relative humidities are controlled to ± 0.1°C and 1% RH of constant or time-varying set points. Minimum temperature is 1 to 3°C above ambient. Total cost was $140,000 for 56 chambers, excluding labor to develop and test the chambers and software.

The objective of this work was to design, construct, and test a set of six ECs suitable for investigating the effects of temperature on the development of gypsy moth, *Lymantria dispar* L., eggs. Requirements were that each chamber be independently controlled for temperature and capable of a constant (± <1°C) temperature from -10 to 35°C. Only a single photoperiod was required for the set ECs. Time-varying temperature regimes were not required for the experiments described in the following chapters, but deemed a desirable option. It was also required that the total cost of the system not exceed approximately $6,000. Because gypsy moth eggs were the experimental material of interest, it was not required that each chamber have a large internal volume.

Materials and Methods

Chamber design and construction. Initial design plans were partially dictated by budget limitations and currently available equipment: a Keithley System 570 DACS (Keithley Instruments, Inc., Cleveland, OH) and Neslab CC-60 immersion cooler (Neslab Instruments, Newington, NH), and an IBM 8088 with 640K RAM, 20 mbyte harddrive, VGA card and color monitor. The CC-60 was replaced by a Neslab PBC-75 in the final product. A 2 gallon Nalgene tank (Fisher No. 02-961-55A) serves as a reservoir for ethyl alcohol. The reservoir is housed in a plywood box above the chambers and is

insulated with two inches of high density Styrofoam insulation on all sides. Openings in the reservoir enclosure were cut to insert the cooling probe of the immersion cooler and a coolant feed and return line. The single gravity feed line from the reservoir fills a 20 x 2.5 cm (length x dia) manifold made of copper tubing which in turn feeds each of a set of six chambers. Coolant flow to each chamber is controlled by a low-temperature solenoid valve (Asco No. 8263A210) located between the manifold and chamber.

Within each chamber coolant circulates through a 19 x 23 x 1.5 cm (h x d x w) aluminum block that serves as a heat exchanger. Four 0.635 cm holes were drilled at equal spacing through the depth of the block and the ends of each hole were tapped to accept a tubing flange. Three-quarter inch C-Flex tubing (Cole-Parmer No. 6424) joins adjacent flanges, thereby routing the coolant through the block 4 times. Coolant flows to a single return line outside the chambers and is returned to the reservoir by a peristaltic pump (Cole-Parmer No. N07553-20). Three-quarter inch C-Flex tubing is used for feed and return lines. One inch of high density Styrofoam insulation covers the back of the set of chambers and coolant lines and manifold are embedded in channels cut in the insulation. An outer layer of two inches of Styrofoam insulation covering the lines and manifold is removable to permit inspection and replacement of lines.

Chambers are constructed in two tiers of three chambers each. Each tier is constructed of half-inch plywood and measures 160 x 38.1 x 40.6 cm (w x d x h) externally. Two inches of high density Styrofoam insulation on all inside surfaces and chamber partitions of two inch thick Styrofoam creates four 33.0 x 25.4 x 30.5 cm (w x d x h) chambers. Inside each chamber 35W of heat is supplied by a 75W, 240V cartridge heater (C-301/240, Omega Engineering, Stamford, CT), temperature is sensed by a copper-constantan (Type T) thermocouple (5TC-GG-T-20-36, Omega Engineering), light is provided by two 7.5V, 0.22A light bulbs (type 51, Sylvania, Westfield, IN) and

air is circulated by a Peewee Boxer Fan (3115FS-12T-B20, IMC Magnetics Corp., Manchester, NH).

The DACS is equipped with an analog input module with a cold-junction reference (AIM7, Keithley Instruments, Cleveland, OH), permitting direct connection of the chamber thermocouples. Heaters and coolant solenoid valves and pump are connected to a 114V power source via solid state relays (OAC1, Keithley Instruments). The 13 relays are in a 16 channel relay board (PCM3, Keithley Instruments), which is connected to the DACS. Fans are connected to a 114V power source and controlled by toggle switches mounted on the side of one quartet of chambers. Lights are connected to a 114V power source via a transformer (P5015, Stancor Products, Logansport, IN) and controlled by a household timer. Each fan, light, and heater circuit is equipped with a 5, 5, or 10 A quick burning fuse, respectively. A 450 watt uninterruptable power supply (American Power Conversion, West Kingston, RI) can maintain power to the computer and controlling program for approx. 45 min. in the event of a power failure.

Each chamber is lined with Plexiglas panels on the bottom and sides. Eight removable Plexiglas shelves in each chamber are supported by machined groves in the aluminum block and by Plexiglas runners glued to the vertical surfaces of the Plexiglas liners. Maximum humidity is maintained by a 10 x 20 x 3 cm (w x l x d) tray of water in the bottom of each chamber. A specific relative humidity can be maintained by the use of a saturated salt solution (Young 1967).

Two computer programs were written in interpretative BASIC for temperature control. Constant temperature regimes are produced in all chambers by running "constant.bas" (Appendix A) and supplying at run time the starting date and time, and the set temperatures for each chamber. Individual chambers can be disabled by a set temperature of 999.

Time-varying temperature regimes are produced by running "sinwave.bas" (Appendix B). Prior to start-up the user must load six files containing the Julian date and daily maximum and minimum set temperatures for each chamber. Each file may contain up to 365 entries. File entries are loaded into arrays of daily maximum and minimum set temperatures. At start-up the user is asked for the starting Julian day and time. A modified sine wave routine (Allen 1976) regularly alters the set temperature to produce a time-varying temperature regime. A maximum set temperature of 999 turns off the chamber for the day. A minimum set temperature of 999 turns off the chamber at noon of the previous day for 24 h. Equal maximum and minimum set temperatures result in a constant temperature regime, thus allowing a mixed pattern of constant and time varying regimes within the set of chambers.

A status screen on the computer monitor digitally displays the maximum and minimum set temperature, or constant set temperature, and actual temperature of each chamber. The on/off status of heating and cooling devices (solenoid valves) in each chamber are also displayed. At the start of each 5 second cycle the DACS is instructed by the control program to return chamber temperatures to the controlling program where each is compared to the appropriate set temperature. Differences between actual and set temperature in excess of tolerances result in an instruction to the DACS to close the appropriate relay(s) which will turn on a heater, or open a valve and turn on the pump, to heat or cool individual chambers as needed. Differences within tolerances result in the opening of the relay and the deactivation of the heating and cooling devices. Uneven tolerances were used around the set point temperature to avoid conflict between heating and cooling devices within a chamber . Tolerances of 0.25^{o}C above and 1.0^{o}C below set temperature were used. Actual temperatures in each chamber and the reservoir are displayed graphically for 6 h periods. Chamber and reservoir temperatures are averaged

for each 15 min. period and stored in RAM. At the end of the 6 h period the 15-minute-average temperatures are written to the computer hard drive, the screen is refreshed and updated with a new 6 h time scale and date (if necessary), and RAM is cleared for the next 6 h period.

The ECs are kept in a 2.5 x 4.3 m room with ambient temperature maintained at approx. 20°C by an air conditioner.

Chamber testing. Chamber performance was examined under constant temperature regimes over a 39 d period at set temperatures of 5, 10, 15, 20, 25, and 30°C. Frequency distributions in 0.25°C classes were calculated for the 4,320 recorded 15-minute-averages for each chamber. Within each chamber photophase and scotophase temperatures were compared by a two-sided t-test (Zar 1984).

Chamber performance was examined under mixed time-varying and constant temperature regimes over a 33 d period. Three chambers were programmed to emulate summer, autumn, and winter temperatures in Virginia. The pattern of 15-minute-averages for each chamber were compared graphically to the time-varying set temperature for the regime. A fourth chamber was programmed to maintain a constant temperature of 15°C. Frequency distributions in 0.25°C classes were calculated for the 3,168 recorded 15-minute-averages for the constant temperature chamber.

Results

During the 39 d test of constant temperature regimes, means (± S.E.) of the 15-minute-average temperatures in the 5, 10, 15, 20, 25, and 30°C chambers were 4.91 (0.0058), 9.75 (0.0053), 14.76 (0.0056), 19.82 (0.0053), 24.73 (0.0047), and 29.68°C (0.0053), respectively. Fifteen-minute-average temperatures were significantly different

($p<0.05$) between scotophase and photophase in the 5 (4.87 vs 4.92), 10 (9.70 vs 9.75), 15 (14.75 vs 14.77), and 20°C (19.77 vs 19.80) chambers. However, mean differences between the light phases were less than 0.05°C. There were no significant differences between photophase and scotophase temperatures in the 25 or 30°C chambers. Sixty-five percent of the 15-minute average temperatures were within 0.5°C of set temperature (Fig. 2.1). Ninety-four percent of the 15-minute average temperatures were within 0.75°C of set temperature (Fig. 2.1). Only 1% of the fifteen-minute average temperatures in the 30°C chamber differed from the set temperature by more than 1°C. Maximum difference never exceeded 1.10°C.

In the 33 d test of mixed time-varying and constant temperature regimes, the pattern of 15-minute-average temperatures within the three chambers programmed to simulate Virginia temperatures conformed to calculated set temperatures very closely (Figs. 2.2, 2.3). Daily temperature ranges of greater than 20°C were successfully emulated. The mean (± S.E.) of the 15-minute average temperatures from the 15°C constant temperature chamber was 14.86 (0.0057) °C. Eighty-three percent of the 15-minute average temperatures were within 0.5°C of set temperature (Fig. 2.4).

Discussion

The six temperature chambers have operated without failure for approximately four months. During that time they were used to produce constant temperature regimes ranging from -10 to 35°C (30 and 15 degrees below and above room temperature, respectively). At any time the set temperature range among the six chambers varied a maximum of 35°C. The small internal volume, together with good air circulation results in negligible temperature variation within a chamber.

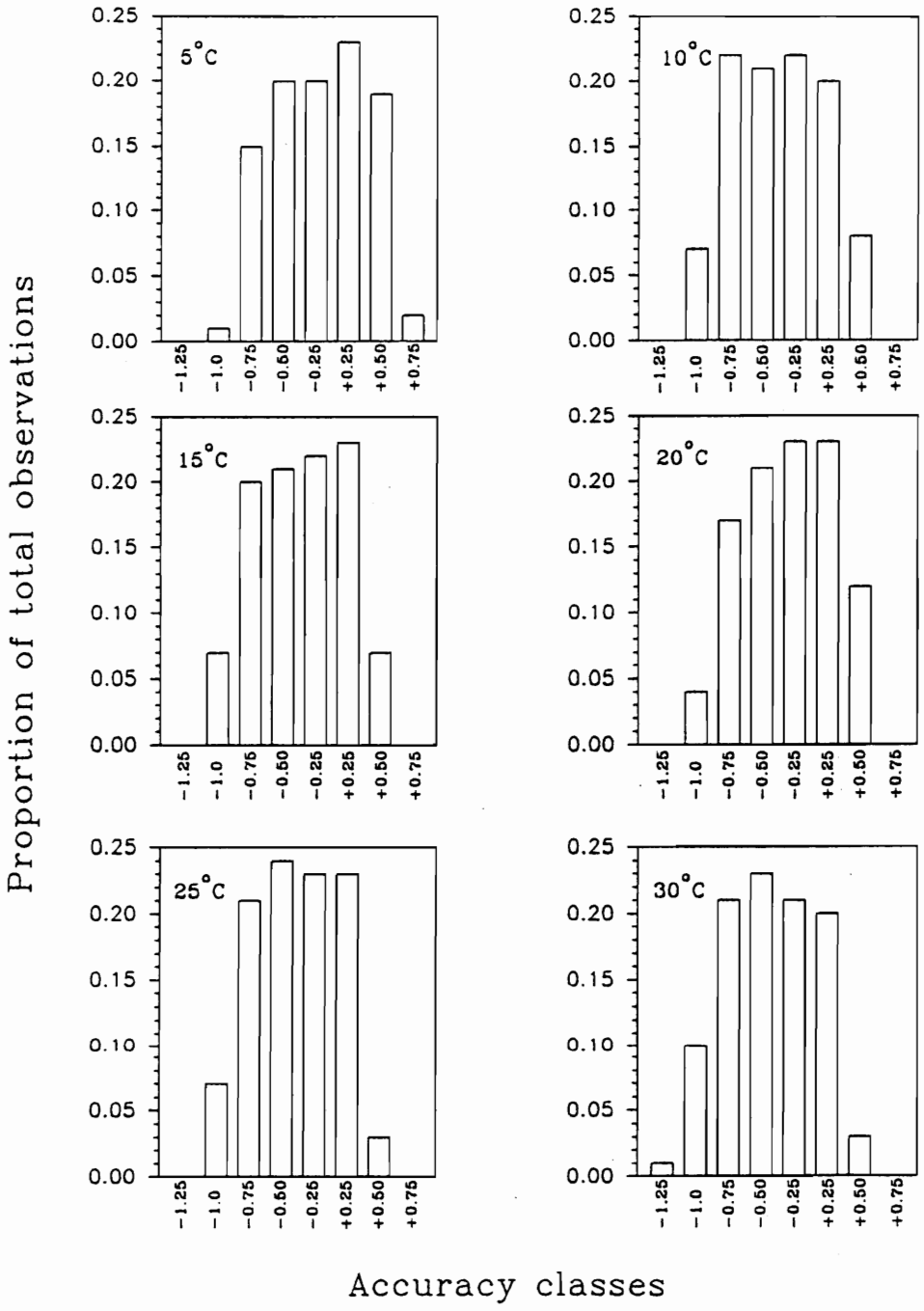

Fig. 2.1. Proportional frequency distributions of 15-minute-average temperatures in 0.25°C accuracy classes for six chambers operating under constant temperature regimes.

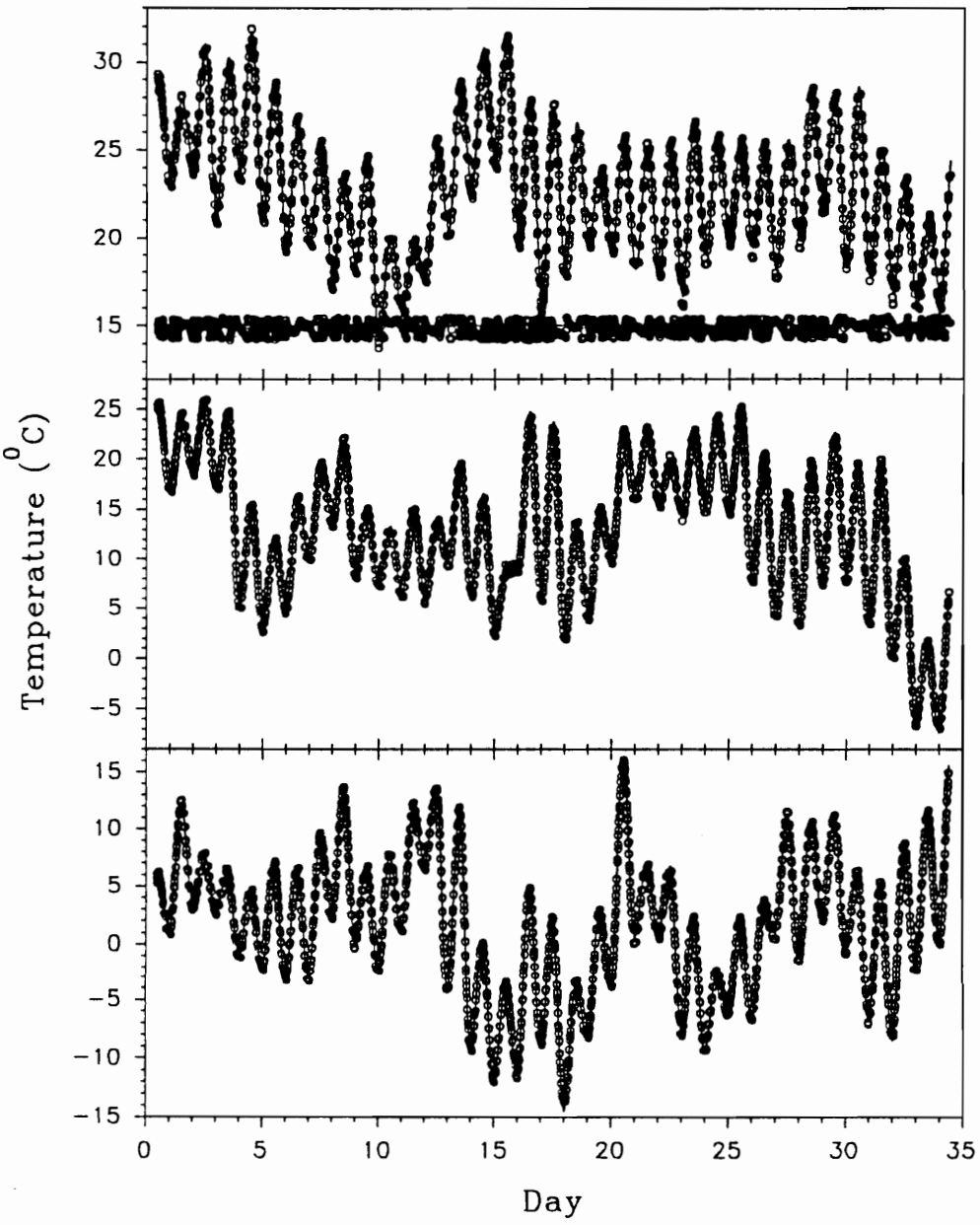

Fig. 2.2. Fifteen-minute average temperatures (o) and sine wave calculated set temperatures (——) for 3 chambers operating under time-varying temperature regimes emulating summer (top), autumn (middle), and winter (bottom) temperatures in Virginia. The top figure shows a time trace of fifteen-minute average temperatures from a chamber operating under a constant temperature regime.

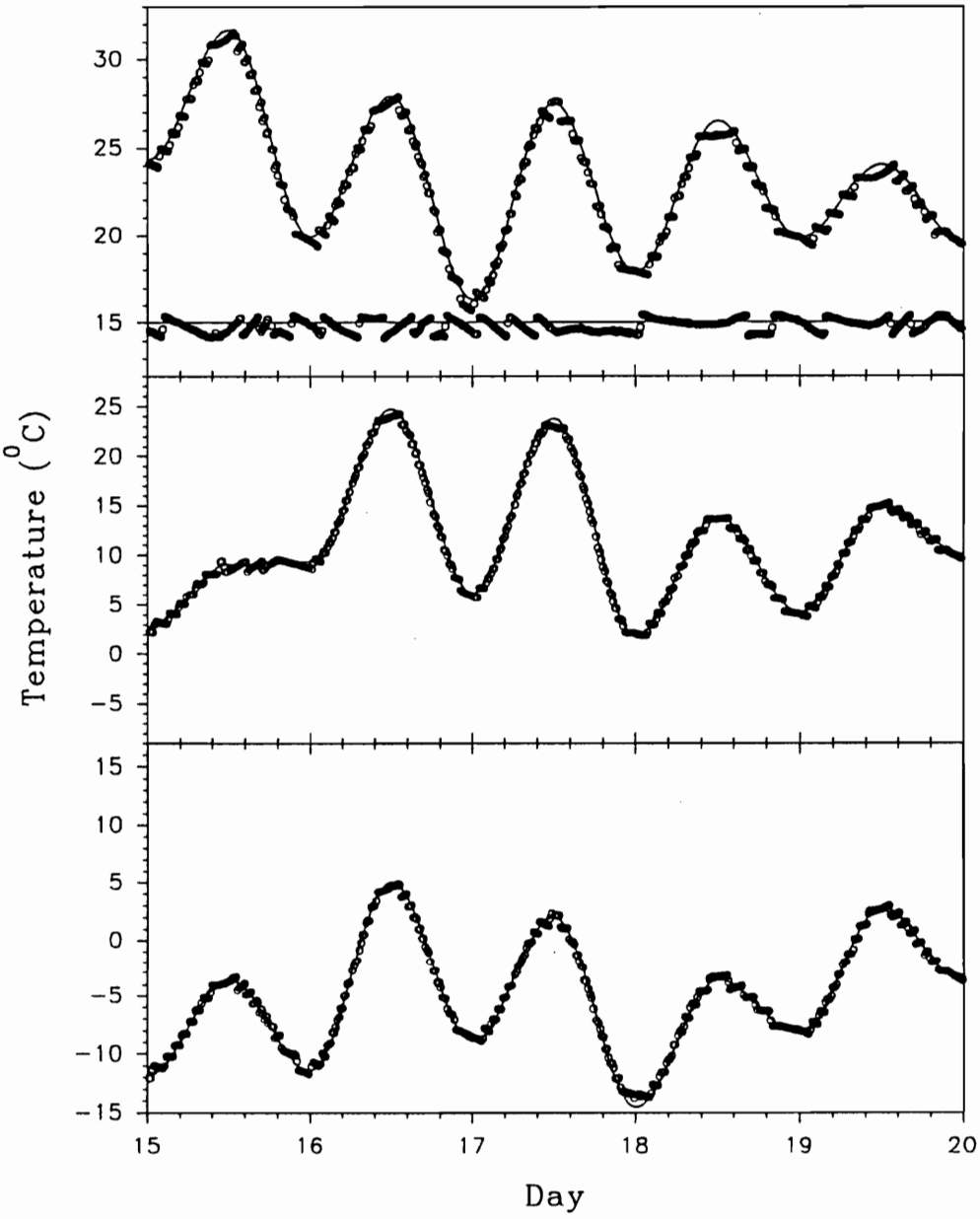

Fig. 2.3. Five days of the temperature trace shown in Fig. 2.

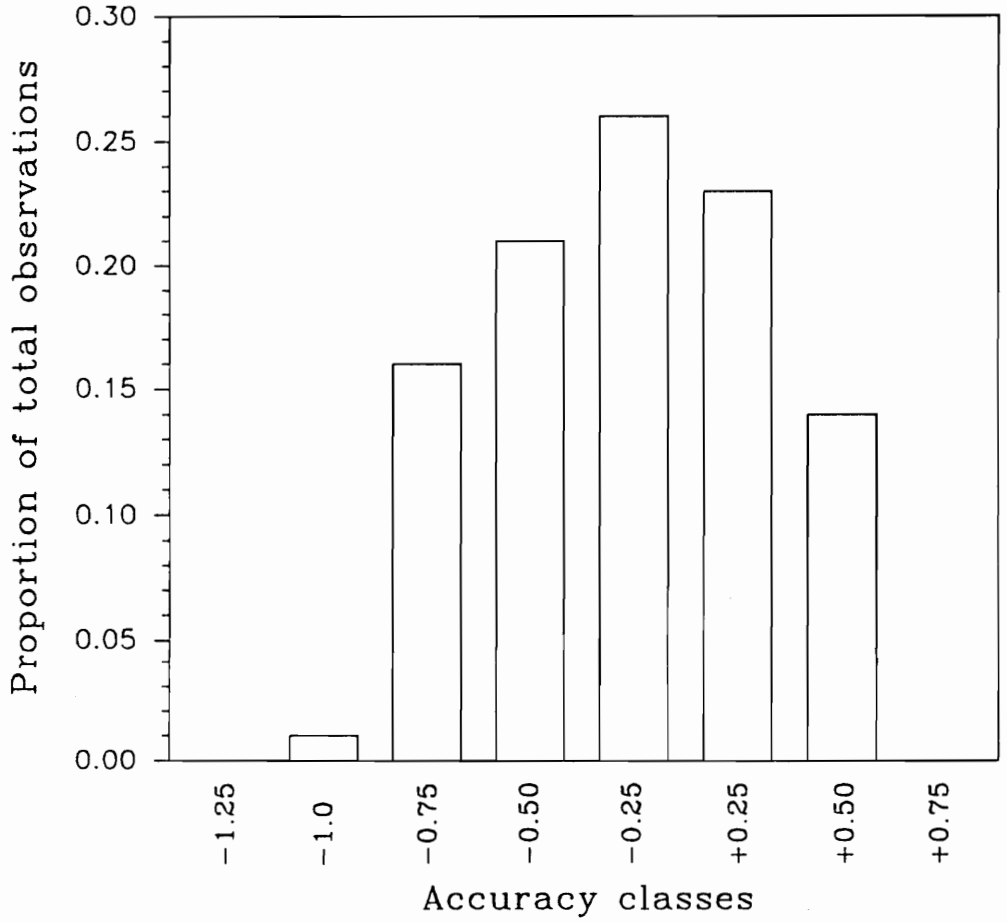

Fig. 2.4. Proportional frequency distributions of 15-minute-average temperatures in 0.25°C accuracy classes for a chamber operating under a constant temperature regime while three companion chambers operated under time-varying regimes.

Ice accumulation on heat exchangers in chambers with subzero temperatures has not been a serious problem. The hard, smooth surface of the heat exchanger permits quick removal of accumulated ice with a heated paint scraper every two to three weeks.

The chambers have performed well when operating for extended periods under either constant, time-varying, or mixed regimes. Operating under constant temperature regimes, mean chamber temperatures differed from set temperatures by -0.32 to -0.09°C in the 5 and 30°C chambers respectively. Deviations from set temperatures were negative in all chambers and averaged -0.23°C.

Attempts to improve chamber accuracy by adding a self-adjustment algorithm to the constant temperature control program were unsuccessful. Addition of the algorithm decreased the precision of chamber temperatures without a noticeable improvement in accuracy. The consistently negative difference between set and 15-minute-average temperatures suggests that greater accuracy could be achieved by a shorter cycling period. This should result in a weaker cooling response when actual temperatures exceed set temperatures. Increasing the positive tolerance on control would also improve temperature accuracy, but would adversely affect temperature precision.

Approximately 94% of the recorded 15-minute-average temperatures were within 0.75°C of the corresponding set temperature, thereby providing satisfactory precision. Less than 0.2% of all 15-minute-average temperatures differed from set temperature by more than 1.0°C.

The minor differences between set and mean temperatures are not excessively large for most experimental purposes. In addition, the choice of any particular set temperature is largely arbitrary. It is often more important that a researcher have an accurate record of the actual temperatures than that the actual temperatures equal the set temperatures.

A closer examination of chamber performance under time-varying regimes (Fig. 3) reveals minor deviations from set temperatures. Deviations were greatest at temperature maxima and minima, but never exceeded 0.75°C. The precision of the constant 15°C chamber was not adversely affected by the time-varying regimes of the other chambers (Fig. 4).

Total expenditure for the six chambers, excluding pre-existing equipment (computer, monitor and DACS) was $5978 (Table 1). The cost of the computer and monitor has not been included since they are usually readily available as surplus items at low cost.

The ECs described here have performed very well. Their reliability, flexible temperature regimes, low cost, and wide temperature range should make them useful for a variety of experiments where large volume is not essential.

Table 1. Itemized list of environmental chamber components and their cost.

Component	Quantity	Unit price	Total
PC c/w 20M hardrive, VGA card and color monitor	1		
immersion cooler	1	1695	1695
DACS	1	1800	1800
input card	1	1020	1020
relay board	1	2250	225
relay	13	25	325
thermocouple	6	7	42
valve	6	125	750
pump	1	225	225
heater	6	25	150
fan	6	15	90
heat exchanger	6	100	600
power backup	1	350	350
tubing	50 ft	58/25ft	116
insulation	4 x 4ft x 8ft	10	40
miscellaneous			350
TOTAL			7778

References Cited

Allen, J. C. 1976. A modified sine wave method for calculating degree days. Environ. Entomol. 5(3):388-396.

Atmar, J. W. , and J. J. Elkington. 1973. An advanced environmental chamber control system. Environ. Entomol. 2(1):88-94.

Roltsch, W. J. ,M. A. Mayse, and K. Clausen. 1990. Temperature-dependent development under constant and fluctuating temperatures: Comparison of linear versus nonlinear methods for modeling development of western grapeleaf skeletonizer (Lepidoptera: Zygaenidae). Environ. Entomol. 19(6):1689-1697.

Tanigoshi, L. K., S. C. Hoyt, R. W. Browne, and J. A. Logan. 1975. Influence of temperature on population increase of Tetranychus mcdaniela (Acarina: Tetranychidae). Ann. Entomol. Soc. Am. 68:972-978.

Walker, T. J., J. J. Gaffney, A. W. Kidder, and A. B. Ziffer. 1993. Florida Reach-ins: Environmental chambers for entomological research. Amer. Entomol. 177-182.

Wilson, K. G., and R. E. Stinner. 1981. Inexpensive, multiple-unit environmental chambers with temperature and humidity control. Ann. Entomol. Soc. Amer. 74:560-562.

Young, J. F. 1967. Humidity control in the laboratory using salt solutions - a review. J. Appl. Chem. 17: 241-245.

Zar, J. H. 1984. Biostatistical Analysis. Prentice Hall. New Jersey. 718 pp.

Appendix A

```
10'-------------------------------------------------------------------------
15' V4.0 03/25/93; CONSTANT TEMPERATURE REGIMES
20' MODIFIED CONTROL FOR LESS CONFLICT BETWEEN HEATING AND COOLING
COMPONENTS
21' LARGER SCREEN AND OUTPUT TO HARDRIVE
25'-------------------------------------------------------------------------
30  FILE$="C:\SOFT500\OUTPUT\TEMP.DAT"
60'-------------------------------------------------------------------------
65'--Set starting date and starting time-----------------------------------
70  INPUT "STARTING DAY (INTEGER ONLY): ";IDAY
75  INPUT "STARTING TIME (24 HOUR CLOCK): ";ITIME
80  DATE=IDAY+ITIME/24
85'
90'--Set desired temperatures for chambers---------------------------------
95  INPUT "TEMPERATURE SETTING FOR CABINET 1: ";DEG1
100  INPUT "TEMPERATURE SETTING FOR CABINET 2: ";DEG2
105  INPUT "TEMPERATURE SETTING FOR CABINET 3: ";DEG3
110  INPUT "TEMPERATURE SETTING FOR CABINET 4: ";DEG4
125  INPUT "TEMPERATURE SETTING FOR CABINET 5: ";DEG5
130  INPUT "TEMPERATURE SETTING FOR CABINET 6: ";DEG6
135'-------------------------------------------------------------------------
140  CALL INIT
145  CALL WARNOFF
150'
160'--Set I/O names-------------------------------------------------------
170  CALL IONAME'("COLDJUNC",8,32,12)
180'
190  CALL IONAME'("CHAMB_1",8,0,12)
195  CALL IONAME'("CHAMB_2",8,1,12)
200  CALL IONAME'("CHAMB_3",8,2,12)
205  CALL IONAME'("CHAMB_4",8,3,12)
210  CALL IONAME'("CHAMB_5",8,4,12)
215  CALL IONAME'("CHAMB_6",8,5,12)
217  CALL IONAME'("RESVR",8,6,12)
220  CALL IONAME'("C1",7,0)
225  CALL IONAME'("C2",7,2)
230  CALL IONAME'("C3",7,4)
235  CALL IONAME'("C4",7,6)
240  CALL IONAME'("C5",7,8)
245  CALL IONAME'("C6",7,10)
250  CALL IONAME'("H1",7,1)
255  CALL IONAME'("H2",7,3)
260  CALL IONAME'("H3",7,5)
265  CALL IONAME'("H4",7,7)
270  CALL IONAME'("H5",7,9)
275  CALL IONAME'("H6",7,11)
277  CALL IONAME'("PUMP",7,12)
280'-------------------------------------------------------------------------
285'--Initialize variables-------------------------------------------------
```

```
288 TOL=.50                    'Set tolerance range'
290 VZERO=0:VONE=1:VA0#=0:J=0:I=0:PUMP=0
295 RESVR=99:TEMP1=99:TEMP2=99:TEMP3=99:TEMP4=99:TEMP5=99:TEMP6=99
300 RESVROLD=-
30:Y1OLD=99:Y2OLD=99:Y3OLD=99:Y4OLD=99:Y5OLD=99:Y6OLD=99:XOLD=0
305'---------------------------------------------------------------------------
308'--Start the timer/counter and turn interrupts on--------------------------
310 INPUT "SECONDS PER CYCLE: ";CYCLE          'Set cycle time (seconds)'
312 DELAYS=CYCLE/.01          'Number of interupts based on .01 sec interupts'
314 DURATION=21600/CYCLE      'Duration of 6 hr screen (# of cycles)'
318 CALL TIMERSTART'(0)
320'
325 CALL INTON'(10,"MIL")
330'
335'--Set up screen display---------------------------------------------------
340 SCREEN 9                   'Set graphics to high resolution.'
345 FOR J=1 TO 4               'J=6 hours; 4 cycles = 24 hours.'
347   DIM TEMP(6,216):DIM RESTEMP(216)     'Initialize arrays for 6 hr cycle'
350   VIEW (1,1)-(638,339):CLS
352   VIEW (1,5)-(500,85),,1
353   WINDOW (0,0)-(100,100)
360   LINE (0,0)-(0,100),1:LINE (0,100)-(100,100),1:LINE (100,100)-(100,0),1:LINE (100,0)-(0,0),1
365   LINE (0,50)-(100,50),1
370   LOCATE 2,2:PRINT"SET";:LOCATE 3,2:PRINT"TEMP";
375   COLOR 15:LOCATE 5,2:PRINT"HEAT";:LOCATE 6,2:PRINT"COOL";
380   COLOR 14:LOCATE 2,7:PRINT USING"###.#";DEG1;
385   COLOR 11:LOCATE 2,16:PRINT USING"###.#";DEG2;
390   COLOR 9:LOCATE 2,25:PRINT USING"###.#";DEG3;
395   COLOR 10:LOCATE 2,34:PRINT USING"###.#";DEG4;
400   COLOR 12:LOCATE 2,43:PRINT USING"###.#";DEG5;
405   COLOR 13:LOCATE 2,52:PRINT USING"###.#";DEG6;
410   COLOR 15
415   VIEW (1,95)-(638,339),,1
417   WINDOW (-250,-55)-(DURATION,35)
420   LINE (0,-40)-(0,35),15                    'Y AXIS'
425   LINE (0,-40)-(DURATION,-40),15:LOCATE 22,2:PRINT"-40"      'X AXIS'
426   LINE (0,-30)-(DURATION,-30),15,,&HCCC:LOCATE 20,2:PRINT"-30"  '-30 DEG
REFERENCE LINE'
430   LINE (0,-20)-(DURATION,-20),15,,&HCCC:LOCATE 18,2:PRINT"-20"  '-20 DEG
REFERENCE LINE'
435   LINE (0,-10)-(DURATION,-10),15,,&HCCC:LOCATE 16,2:PRINT"-10"  '-10 DEG
REFERENCE LINE'
440   LINE (0,0)-(DURATION,0),15,,&HCCC:LOCATE 14,4:PRINT"0"      '0 DEG REFERENCE
LINE'
445   LINE (0,10)-(DURATION,10),15,,&HCCC:LOCATE 12,3:PRINT"10"    '10 DEG REFERENCE
LINE'
450   LINE (0,20)-(DURATION,20),15,,&HCCC:LOCATE 10,3:PRINT"20"    '20 DEG REFERENCE
LINE'
455   LINE (0,30)-(DURATION,30),15,,&HCCC:LOCATE 8,3:PRINT"30"     '30 DEG REFERENCE
LINE'
460'
```

```
465   LOCATE 23,4:PRINT USING"##.##";(ITIME+(J-1)*6) MOD 24;
470   LOCATE 23,23:PRINT USING"##.##";(ITIME+(J-1)*6) MOD 24+1.5;
475   LOCATE 23,42:PRINT USING"##.##";(ITIME+(J-1)*6) MOD 24+3.0;
480   LOCATE 23,61:PRINT USING"##.##";(ITIME+(J-1)*6) MOD 24+4.5;
485   LOCATE 24,33:PRINT "JULIAN DATE:";:PRINT INT(DATE);
490'
495'-------------------------------------------------------------------------
500'--Begin timed temperature control loop-----------------------------------
505   FOR I=1 TO DURATION          '# of cycles per 6 hr screen'
510     CALL TIMERREAD'(0,VA0#)
515     IF VA0# < DELAYS*I GOTO 510      'DELAYS=no. of interupts for cycle freq.'
520       LOCATE 2,65:PRINT I:LOCATE 3,65:PRINT VA0#/DELAYS
525       X=I+1                    'Increment X variable for graph'
530'
535'  This next line causes K to have the same value for repeated cycles
540'  such that all but the last value is overwritten and only one
545'  value per 15 minutes is saved. (The mean temperature is saved)
547       REP=60*15/CYCLE
550       K=INT(I/REP)
555'
560       XOLD=X          'Resets x axis (time) value.'
565'
570'----------Temperature control routines-------------------------------------
572'--Reads temperature, prints temp to screen, draws line, calls control.
574'
576       PUMP=0             'Default pump setting is OFF'
578       CALL ANREAD'("RESVR",RESVR,13)
580       RESTEMP(K)=RESVR:LOCATE 4,65:PRINT USING"###.#";RESVR
582       LINE(XOLD,RESVROLD)-(X,RESVR),5
584       RESVROLD=RESVR
585'---Chamber 1----------------------------------------------------------------
590       IF DEG1 >= 99 GOTO 650
592       COLOR 14
594       CALL ANREAD'("CHAMB_1",TEMP1,13)
596       TEMP(1,K)=TEMP(1,K)+TEMP1/REP
598       LOCATE 3,7:PRINT USING"###.##";TEMP1;
600       LINE (XOLD,Y1OLD)-(X,TEMP1)
602       Y1OLD=TEMP1
618       IF TEMP1 >= DEG1+.25 THEN GOSUB 1120: GOTO 650
620       IF TEMP1 > DEG1-1.25 AND TEMP1 < DEG1+.25 THEN GOSUB 1155: GOTO 650
622       IF TEMP1 <= DEG1-1.25 THEN GOSUB 1190: GOTO 650
640'
645'---Chamber 2----------------------------------------------------------------
650       IF DEG2 >= 99 GOTO 710
652       COLOR 11
654       CALL ANREAD'("CHAMB_2",TEMP2,13)
656       TEMP(2,K)=TEMP(2,K)+TEMP2/REP
658       LOCATE 3,16:PRINT USING"###.##";TEMP2;
660       LINE (XOLD,Y2OLD)-(X,TEMP2)
662       Y2OLD=TEMP2
678       IF TEMP2 >= DEG2+.25 THEN GOSUB 1235: GOTO 710
```

```
680         IF TEMP2 > DEG2-1.25 AND TEMP2 < DEG2+.25 THEN GOSUB 1270: GOTO 710
682         IF TEMP2 <= DEG2-1.25 THEN GOSUB 1305: GOTO 710
700'
705'---Chamber 3-------------------------------------------------------------
710         IF DEG3 >= 99 GOTO 770
712         COLOR 9
714         CALL ANREAD'("CHAMB_3",TEMP3,13)
716         TEMP(3,K)=TEMP(3,K)+TEMP3/REP
718         LOCATE 3,25:PRINT USING"###.##";TEMP3;
720         LINE (XOLD,Y3OLD)-(X,TEMP3)
722         Y3OLD=TEMP3
738         IF TEMP3 >= DEG3+.25 THEN GOSUB 1350: GOTO 770
740         IF TEMP3 > DEG3-1.25 AND TEMP3 < DEG3+.25 THEN GOSUB 1385: GOTO 770
742         IF TEMP3 <= DEG3-1.25 THEN GOSUB 1420: GOTO 770
760'
765'---Chamber 4-------------------------------------------------------------
770         IF DEG4 >= 99 GOTO 830
772         COLOR 10
774         CALL ANREAD'("CHAMB_4",TEMP4,13)
776         TEMP(4,K)=TEMP(4,K)+TEMP4/REP
778         LOCATE 3,34:PRINT USING"###.##";TEMP4;
780         LINE (XOLD,Y4OLD)-(X,TEMP4)
782         Y4OLD=TEMP4
798         IF TEMP4 >= DEG4+.25 THEN GOSUB 1465: GOTO 830
800         IF TEMP4 > DEG4-1.25 AND TEMP4 < DEG4+.25 THEN GOSUB 1500: GOTO 830
802         IF TEMP4 <= DEG4-1.25 THEN GOSUB 1535: GOTO 830
820'
825'---Chamber 5-------------------------------------------------------------
830         IF DEG5 >= 99 GOTO 890
832         COLOR 12
834         CALL ANREAD'("CHAMB_5",TEMP5,13)
836         TEMP(5,K)=TEMP(5,K)+TEMP5/REP
838         LOCATE 3,43:PRINT USING"###.##";TEMP5;
840         LINE (XOLD,Y5OLD)-(X,TEMP5)
842         Y5OLD=TEMP5
858         IF TEMP5 >= DEG5+.25 THEN GOSUB 1580: GOTO 890
860         IF TEMP5 > DEG5-1.25 AND TEMP5 < DEG5+.25 THEN GOSUB 1615: GOTO 890
862         IF TEMP5 <= DEG5-1.25 THEN GOSUB 1650: GOTO 890
880'
885'---Chamber 6-------------------------------------------------------------
890         IF DEG6 >= 99 GOTO 990
892         COLOR 13
894         CALL ANREAD'("CHAMB_6",TEMP6,13)
896         TEMP(6,K)=TEMP(6,K)+TEMP6/REP
898         LOCATE 3,52:PRINT USING"###.##";TEMP6;
900         LINE (XOLD,Y6OLD)-(X,TEMP6)
902         Y6OLD=TEMP6
918         IF TEMP6 >= DEG6+.25 THEN GOSUB 1695: GOTO 990
920         IF TEMP6 > DEG6-1.25 AND TEMP6 < DEG6+.25 THEN GOSUB 1730: GOTO 990
922         IF TEMP6 <= DEG6-1.25 THEN GOSUB 1765: GOTO 990
985'-------------------------------------------------------------------------
```

```
990       IF PUMP => 1 GOTO 1010          'Check pump setting'
995       CALL DIGWRITE'("PUMP",VZERO)
1000'
1010       COLOR 15
1015       IF INKEY$ = "I" GOTO 1910
1020'      IF INKEY$ <>"" GOTO 1910
1030   NEXT I
1032'---Restart timer for new 6 hour cycle
1035   CALL TIMERSTART'(0)
1040   GOSUB 1820          'Dump data to buffer before start of next 6 hour cycle.'
1045   CLOSE #1            'Closes file in order to dump buffer to disk.'
1050   I=1                 'Reset counter for start of next 6 hour cycle.'
1055   DATE=DATE+0.25      'Add 6 hours to the date.'
1057   ERASE TEMP, RESTEMP   'Erase arrays for redeclaration at start of new cycle.'
1060 NEXT J                'Begin next 6 hour cycle.'
1065 J=1                   'Reset counter for start of next 24 hour cycle.'
1070 GOTO 345              'Return to start of 24 hour cycle.'
1080'
1090'--End timed temperature control loop-----------------------------------------
1100'
1115'--Temperature controls for chamber 1-----------------------------------------
1120 CALL DIGWRITE'("H1",VZERO)
1125 LOCATE 5,7:PRINT"      ";
1130 CALL DIGWRITE'("C1",VONE)
1132 CALL DIGWRITE'("PUMP",VONE)
1133 PUMP=PUMP+1
1135 LOCATE 6,7:PRINT"  ON  ";
1140' HEAT(1,I)=0:COOL(1,I)=1
1145 RETURN
1150'
1155 CALL DIGWRITE'("H1",VZERO)
1160 LOCATE 5,7:PRINT"      ";
1165 CALL DIGWRITE'("C1",VZERO)
1170 LOCATE 6,7:PRINT"      ";
1175' HEAT(1,I)=0:COOL(1,I)=0
1180 RETURN
1185'
1190 CALL DIGWRITE'("H1",VONE)
1195 LOCATE 5,7:PRINT"  ON  ";
1200 CALL DIGWRITE'("C1",VZERO)
1205 LOCATE 6,7:PRINT"      ";
1210' HEAT(1,I)=1:COOL(1,I)=0
1215 RETURN
1220'-----------------------------------------------------------------------------
1225'
1230'--Temperature controls for chamber 2-----------------------------------------
1235 CALL DIGWRITE'("H2",VZERO)
1240 LOCATE 5,16:PRINT"      ";
1245 CALL DIGWRITE'("C2",VONE)
1247 CALL DIGWRITE'("PUMP",VONE)
1248 PUMP=PUMP+1
```

```
1250 LOCATE 6,16:PRINT"   ON  ";
1255' HEAT(2,I)=0:COOL(2,I)=1
1260 RETURN
1265'
1270 CALL DIGWRITE'("H2",VZERO)
1275 LOCATE 5,16:PRINT"       ";
1280 CALL DIGWRITE'("C2",VZERO)
1285 LOCATE 6,16:PRINT"       ";
1290' HEAT(2,I)=0:COOL(2,I)=0
1295 RETURN
1300'
1305 CALL DIGWRITE'("H2",VONE)
1310 LOCATE 5,16:PRINT"   ON  ";
1315 CALL DIGWRITE'("C2",VZERO)
1320 LOCATE 6,16:PRINT"       ";
1325' HEAT(2,I)=1:COOL(2,I)=0
1330 RETURN
1335'-------------------------------------------------------------------------
1340'
1345'--Temperature controls for chamber 3-----------------------------------
1350 CALL DIGWRITE'("H3",VZERO)
1355 LOCATE 5,25:PRINT"       ";
1360 CALL DIGWRITE'("C3",VONE)
1362 CALL DIGWRITE'("PUMP",VONE)
1363 PUMP=PUMP+1
1365 LOCATE 6,25:PRINT"   ON  ";
1370' HEAT(3,I)=0:COOL(3,I)=1
1375 RETURN
1380'
1385 CALL DIGWRITE'("H3",VZERO)
1390 LOCATE 5,25:PRINT"       ";
1395 CALL DIGWRITE'("C3",VZERO)
1400 LOCATE 6,25:PRINT"       ";
1405' HEAT(3,I)=0:COOL(3,I)=0
1410 RETURN
1415'
1420 CALL DIGWRITE'("H3",VONE)
1425 LOCATE 5,25:PRINT"   ON  ";
1430 CALL DIGWRITE'("C3",VZERO)
1435 LOCATE 6,25:PRINT"       ";
1440' HEAT(3,I)=1:COOL(3,I)=0
1445 RETURN
1450'-------------------------------------------------------------------------
1455'
1460'--Temperature controls for chamber 4-----------------------------------
1465 CALL DIGWRITE'("H4",VZERO)
1470 LOCATE 5,34:PRINT"       ";
1475 CALL DIGWRITE'("C4",VONE)
1477 CALL DIGWRITE'("PUMP",VONE)
1478 PUMP=PUMP+1
1480 LOCATE 6,34:PRINT"   ON  ";
```

```
1485'  HEAT(4,I)=0:COOL(4,I)=1
1490  RETURN
1495'
1500  CALL DIGWRITE'("H4",VZERO)
1505  LOCATE 5,34:PRINT"      ";
1510  CALL DIGWRITE'("C4",VZERO)
1515  LOCATE 6,34:PRINT"      ";
1520'  HEAT(4,I)=0:COOL(4,I)=0
1525  RETURN
1530'
1535  CALL DIGWRITE'("H4",VONE)
1540  LOCATE 5,34:PRINT"  ON  ";
1545  CALL DIGWRITE'("C4",VZERO)
1550  LOCATE 6,34:PRINT"      ";
1555'  HEAT(4,I)=1:COOL(4,I)=0
1560  RETURN
1565'-------------------------------------------------------------------------
1570'
1575'--Temperature controls for chamber 5-----------------------------------
1580  CALL DIGWRITE'("H5",VZERO)
1585  LOCATE 5,43:PRINT"      ";
1590  CALL DIGWRITE'("C5",VONE)
1592  CALL DIGWRITE'("PUMP",VONE)
1593  PUMP=PUMP+1
1595  LOCATE 6,43:PRINT"  ON  ";
1600'  HEAT(5,I)=0:COOL(5,I)=1
1605  RETURN
1610'
1615  CALL DIGWRITE'("H5",VZERO)
1620  LOCATE 5,43:PRINT"      ";
1625  CALL DIGWRITE'("C5",VZERO)
1630  LOCATE 6,43:PRINT"      ";
1635'  HEAT(5,I)=0:COOL(5,I)=0
1640  RETURN
1645'
1650  CALL DIGWRITE'("H5",VONE)
1655  LOCATE 5,43:PRINT"  ON  ";
1660  CALL DIGWRITE'("C5",VZERO)
1665  LOCATE 6,43:PRINT"      ";
1670'  HEAT(5,I)=1:COOL(5,I)=0
1675  RETURN
1680'-------------------------------------------------------------------------
1685'
1690'--Temperature controls for chamber 6-----------------------------------
1695  CALL DIGWRITE'("H6",VZERO)
1700  LOCATE 5,52:PRINT"      ";
1705  CALL DIGWRITE'("C6",VONE)
1707  CALL DIGWRITE'("PUMP",VONE)
1708  PUMP=PUMP+1
1710  LOCATE 6,52:PRINT"  ON  ";
1715'  HEAT(6,I)=0:COOL(6,I)=1
```

```
1720 RETURN
1725'
1730 CALL DIGWRITE'("H6",VZERO)
1735 LOCATE 5,52:PRINT"      ";
1740 CALL DIGWRITE'("C6",VZERO)
1745 LOCATE 6,52:PRINT"      ";
1750' HEAT(6,I)=0:COOL(6,I)=0
1755 RETURN
1760'
1765 CALL DIGWRITE'("H6",VONE)
1770 LOCATE 5,52:PRINT"  ON  ";
1775 CALL DIGWRITE'("C6",VZERO)
1780 LOCATE 6,52:PRINT"      ";
1785' HEAT(6,I)=1:COOL(6,I)=0
1790 RETURN
1795'-------------------------------------------------------------------------
1800'
1810'--Write temperature arrays to disk at end of each 6 hour loop---------------------
1820 LOCATE 20,20:PRINT "END OF 6 HOUR LOOP; DUMPING"
1825 OPEN FILE$ FOR APPEND AS #1
1830 L=1
1840 FOR L=1 TO K-1
1850   PRINT #1,USING"###.### ";DATE+L*REP*CYCLE/86400;
1855   PRINT #1,USING"###.##
";RESTEMP(L);TEMP(1,L);TEMP(2,L);TEMP(3,L);TEMP(4,L);TEMP(5,L);TEMP(6,L)
1860 NEXT L
1870 RETURN
1880'-------------------------------------------------------------------------
1890'
1900'--Write temperature arrays to disk if program interrupted----------------
1910 LOCATE 20,20:PRINT "INTERRUPT SUB; DUMPING"
1915 OPEN FILE$ FOR APPEND AS #1
1920 L=1
1930 FOR L=1 TO K-1
1940   PRINT #1,USING"###.### ";DATE+L*REP*CYCLE/86400;
1945   PRINT #1,USING"###.##
";RESTEMP(L);TEMP(1,L);TEMP(2,L);TEMP(3,L);TEMP(4,L);TEMP(5,L);TEMP(6,L)
1950 NEXT L
1960'-------------------------------------------------------------------------
1970'
1980'--Turn off interrupts and turn off all temperature controls-------------
1990 CALL INTOFF
2000'
2010 CALL DIGWRITE'("C1",VZERO)
2020 CALL DIGWRITE'("H1",VZERO)
2030 CALL DIGWRITE'("C2",VZERO)
2040 CALL DIGWRITE'("H2",VZERO)
2050 CALL DIGWRITE'("C3",VZERO)
2060 CALL DIGWRITE'("H3",VZERO)
2070 CALL DIGWRITE'("C4",VZERO)
2080 CALL DIGWRITE'("H4",VZERO)
```

```
2090 CALL DIGWRITE'("C5",VZERO)
3000 CALL DIGWRITE'("H5",VZERO)
3010 CALL DIGWRITE'("C6",VZERO)
3020 CALL DIGWRITE'("H6",VZERO)
3025 CALL DIGWRITE'("PUMP",VZERO)
3030 END
```

Appendix B

```
5'-------------------------------------------------------------------------
10'  V5.02 08/17/93; SINEWAVE TEMPERATURE REGIMES
15'-------------------------------------------------------------------------
20  FILEOUT$="C:\SOFT500\OUTPUT\TEMP.DAT"
21  COLOR 12
22  CLS
23  PRINT"     ************************************************************"
24  PRINT"     ******         HOKIE TEMPERATURE SIMULATOR          ******"
25  PRINT"     ****** SOFTWARE BY KEITHLEY INSTRUMENTS (CLEVELAND, OH) ******"
26  PRINT"     ******      AND DAVID GRAY (VIRGINIA POLYTECH)      ******"
27  PRINT"     ******           (703) 231-4161                 ******"
28  PRINT"     ******            VERSION 5.0                   ******"
29  PRINT"     ************************************************************"
35'-------------------------------------------------------------------------
40'--Set starting date and starting time-----------------------------------
42  INPUT "STARTING DAY (INTEGER ONLY): ";DAY
43  INPUT "STARTING TIME (##.## eg 13.50 for 1:30 PM): ";ITIME
44  DATE=DAY+ITIME/24
46'
47'--Set desired temperatures for chambers----------------------------------
50  DIM MIN1(365):DIM MIN2(365):DIM MIN3(365):DIM MIN4(365):DIM MIN5(365):DIM
MIN6(365)
55  DIM MAX1(365):DIM MAX2(365):DIM MAX3(365):DIM MAX4(365):DIM MAX5(365):DIM
MAX6(365)
60  FILEIN$="C:\SOFT500\INPUT\TEMP1.MXM": OPEN FILEIN$ FOR INPUT ACCESS READ AS
#1
61  FOR I=1 TO 365
62    IF EOF(1) THEN 65
63    INPUT#1,FILEDAY,MIN1(FILEDAY),MAX1(FILEDAY)
64  NEXT I
65  CLOSE #1
70  FILEIN$="C:\SOFT500\INPUT\TEMP2.MXM": OPEN FILEIN$ FOR INPUT ACCESS READ AS
#1
71  FOR I=1 TO 365
72    IF EOF(1) THEN 75
73    INPUT#1,FILEDAY,MIN2(FILEDAY),MAX2(FILEDAY)
74  NEXT I
75  CLOSE #1
80  FILEIN$="C:\SOFT500\INPUT\TEMP3.MXM": OPEN FILEIN$ FOR INPUT ACCESS READ AS
#1
81  FOR I=1 TO 365
82    IF EOF(1) THEN 85
83    INPUT#1,FILEDAY,MIN3(FILEDAY),MAX3(FILEDAY)
84  NEXT I
85  CLOSE #1
90  FILEIN$="C:\SOFT500\INPUT\TEMP4.MXM": OPEN FILEIN$ FOR INPUT ACCESS READ AS
#1
91  FOR I=1 TO 365
92    IF EOF(1) THEN 95
```

```
93    INPUT#1,FILEDAY,MIN4(FILEDAY),MAX4(FILEDAY)
94  NEXT I
95  CLOSE #1
100  FILEIN$="C:\SOFT500\INPUT\TEMP5.MXM": OPEN FILEIN$ FOR INPUT ACCESS READ AS
#1
101  FOR I=1 TO 365
102    IF EOF(1) THEN 105
103    INPUT#1,FILEDAY,MIN5(FILEDAY),MAX5(FILEDAY)
104  NEXT I
105 CLOSE #1
110  FILEIN$="C:\SOFT500\INPUT\TEMP6.MXM": OPEN FILEIN$ FOR INPUT ACCESS READ AS
#1
111  FOR I=1 TO 365
112    IF EOF(1) THEN 115
113    INPUT#1,FILEDAY,MIN6(FILEDAY),MAX6(FILEDAY)
114  NEXT I
115  CLOSE #1
138'-------------------------------------------------------------------------
140  CALL INIT
145  CALL WARNOFF
150'
160'--Set I/O names-----------------------------------------------------------
170  CALL IONAME'("COLDJUNC",8,32,12)
180'
190  CALL IONAME'("CHAMB_1",8,0,12)
195  CALL IONAME'("CHAMB_2",8,1,12)
200  CALL IONAME'("CHAMB_3",8,2,12)
205  CALL IONAME'("CHAMB_4",8,3,12)
210  CALL IONAME'("CHAMB_5",8,4,12)
215  CALL IONAME'("CHAMB_6",8,5,12)
217  CALL IONAME'("RESVR",8,6,12)
220  CALL IONAME'("C1",7,0)
225  CALL IONAME'("C2",7,2)
230  CALL IONAME'("C3",7,4)
235  CALL IONAME'("C4",7,6)
240  CALL IONAME'("C5",7,8)
245  CALL IONAME'("C6",7,10)
250  CALL IONAME'("H1",7,1)
255  CALL IONAME'("H2",7,3)
260  CALL IONAME'("H3",7,5)
265  CALL IONAME'("H4",7,7)
270  CALL IONAME'("H5",7,9)
275  CALL IONAME'("H6",7,11)
277  CALL IONAME'("PUMP",7,12)
280'-------------------------------------------------------------------------
285'--Initialize variables----------------------------------------------------
288  TEST1=1:TEST2=1:TEST3=1:TEST4=1:TEST5=1:TEST6=1    'Turn chambers test on '
290  VZERO=0:VONE=1:VA0#=0:J=0:I=0:PUMP=0
295  RESVR=99:TEMP1=99:TEMP2=99:TEMP3=99:TEMP4=99:TEMP5=99:TEMP6=99
300  RESVROLD=-
30:Y1OLD=99:Y2OLD=99:Y3OLD=99:Y4OLD=99:Y5OLD=99:Y6OLD=99:XOLD=0
```

```
305'-------------------------------------------------------------------------
308'--Start the timer/counter and turn interrupts on--------------------------
310 CYCLE=5                  'Set cycle time (seconds)'
312 DELAYS=CYCLE/.01         'Number of interupts based on .01 sec interupts'
314 DURATION=21600/CYCLE     '# of cycles of 6 hr screen'
316 START=(ITIME*100 MOD 600)*36/CYCLE   'Starting position on screen for 1st 6 hr cycle'
318 CALL TIMERSTART'(0)
320'
325 CALL INTON'(10,"MIL")
330'
335'--Set up screen display--------------------------------------------------
340 SCREEN 9                 'Set graphics to high resolution.'
345 FOR J=1 TO 4             'J=6 hours; 4 cycles = 24 hours.'
346   DIM TEMP(6,75):DIM RESTEMP(75)      'Initialize arrays for 6 hr cycle'
348   VIEW (1,1)-(638,339):CLS
350   VIEW (1,5)-(500,85),,1
352   WINDOW (0,0)-(100,100)
354   LINE (0,0)-(0,100),1:LINE (0,100)-(100,100),1:LINE (100,100)-(100,0),1:LINE (100,0)-(0,0),1
356   LINE (0,50)-(100,50),1
358   LOCATE 2,2:PRINT"SET";:LOCATE 3,2:PRINT"TEMP";
360   COLOR 15:LOCATE 5,2:PRINT"HEAT";:LOCATE 6,2:PRINT"COOL";
362   IF (DATE-INT(DATE))*24 >= 12 GOTO 390
365     COLOR 14:LOCATE 2,7:PRINT USING"###/###";MIN1(DAY),MAX1(DAY);
366     COLOR 11:LOCATE 2,16:PRINT USING"###/###";MIN2(DAY),MAX2(DAY);
368     COLOR 9:LOCATE 2,25:PRINT USING"###/###";MIN3(DAY),MAX3(DAY);
370     COLOR 10:LOCATE 2,34:PRINT USING"###/###";MIN4(DAY),MAX4(DAY);
372     COLOR 12:LOCATE 2,43:PRINT USING"###/###";MIN5(DAY),MAX5(DAY);
374     COLOR 13:LOCATE 2,52:PRINT USING"###/###";MIN6(DAY),MAX6(DAY);
376     MEAN1=(MIN1(DAY)+MAX1(DAY))/2: RANGE1=(MAX1(DAY)-MIN1(DAY))/2
378     MEAN2=(MIN2(DAY)+MAX2(DAY))/2: RANGE2=(MAX2(DAY)-MIN2(DAY))/2
380     MEAN3=(MIN3(DAY)+MAX3(DAY))/2: RANGE3=(MAX3(DAY)-MIN3(DAY))/2
382     MEAN4=(MIN4(DAY)+MAX4(DAY))/2: RANGE4=(MAX4(DAY)-MIN4(DAY))/2
384     MEAN5=(MIN5(DAY)+MAX5(DAY))/2: RANGE5=(MAX5(DAY)-MIN5(DAY))/2
386     MEAN6=(MIN6(DAY)+MAX6(DAY))/2: RANGE6=(MAX6(DAY)-MIN6(DAY))/2
388     GOTO 414
390   COLOR 14:LOCATE 2,7:PRINT USING"###/###";MAX1(DAY),MIN1(DAY+1);
392   COLOR 11:LOCATE 2,16:PRINT USING"###/###";MAX2(DAY),MIN2(DAY+1);
394   COLOR 9:LOCATE 2,25:PRINT USING"###/###";MAX3(DAY),MIN3(DAY+1);
396   COLOR 10:LOCATE 2,34:PRINT USING"###/###";MAX4(DAY),MIN4(DAY+1);
398   COLOR 12:LOCATE 2,43:PRINT USING"###/###";MAX5(DAY),MIN5(DAY+1);
400   COLOR 13:LOCATE 2,52:PRINT USING"###/###";MAX6(DAY),MIN6(DAY+1);
402   MEAN1=(MAX1(DAY)+MIN1(DAY+1))/2: RANGE1=(MAX1(DAY)-MIN1(DAY+1))/2
404   MEAN2=(MAX2(DAY)+MIN2(DAY+1))/2: RANGE2=(MAX2(DAY)-MIN2(DAY+1))/2
406   MEAN3=(MAX3(DAY)+MIN3(DAY+1))/2: RANGE3=(MAX3(DAY)-MIN3(DAY+1))/2
408   MEAN4=(MAX4(DAY)+MIN4(DAY+1))/2: RANGE4=(MAX4(DAY)-MIN4(DAY+1))/2
410   MEAN5=(MAX5(DAY)+MIN5(DAY+1))/2: RANGE5=(MAX5(DAY)-MIN5(DAY+1))/2
412   MEAN6=(MAX6(DAY)+MIN6(DAY+1))/2: RANGE6=(MAX6(DAY)-MIN6(DAY+1))/2
414   COLOR 15
415   VIEW (1,95)-(638,339),,1
417   WINDOW (-250,-55)-(DURATION,35)
420   LINE (0,-40)-(0,35),15                    'Y AXIS'
```

```
425    LINE (0,-40)-(DURATION,-40),15:LOCATE 22,2:PRINT"-40"         'X AXIS'
426    LINE (0,-30)-(DURATION,-30),15,,&HCCC:LOCATE 20,2:PRINT"-30"   '-30 DEG
REFERENCE LINE'
430    LINE (0,-20)-(DURATION,-20),15,,&HCCC:LOCATE 18,2:PRINT"-20"   '-20 DEG
REFERENCE LINE'
435    LINE (0,-10)-(DURATION,-10),15,,&HCCC:LOCATE 16,2:PRINT"-10"   '-10 DEG
REFERENCE LINE'
440    LINE (0,0)-(DURATION,0),15,,&HCCC:LOCATE 14,4:PRINT"0"        '0 DEG REFERENCE
LINE'
445    LINE (0,10)-(DURATION,10),15,,&HCCC:LOCATE 12,3:PRINT"10"     '10 DEG REFERENCE
LINE'
450    LINE (0,20)-(DURATION,20),15,,&HCCC:LOCATE 10,3:PRINT"20"     '20 DEG REFERENCE
LINE'
455    LINE (0,30)-(DURATION,30),15,,&HCCC:LOCATE 8,3:PRINT"30"      '30 DEG REFERENCE
LINE'
460'
465    LOCATE 23,6:PRINT USING"##";INT((ITIME+(J-1)*6)/6)*6 MOD 24
470    LOCATE 23,23:PRINT USING"##.#";INT((ITIME+(J-1)*6)/6)*6 MOD 24 + 1.5;
475    LOCATE 23,42:PRINT USING"##";INT((ITIME+(J-1)*6)/6)*6 MOD 24 + 3.0;
480    LOCATE 23,61:PRINT USING"##.#";INT((ITIME+(J-1)*6)/6)*6 MOD 24 + 4.5;
482    LOCATE 23,78:PRINT USING"##";INT((ITIME+(J-1)*6)/6)*6 MOD 24 + 6.0;
485    LOCATE 24,33:PRINT "JULIAN DATE:";:PRINT INT(DATE);
490'
495'-------------------------------------------------------------------------
500'--Begin timed temperature control loop-----------------------------------
501    FOR I=1 TO DURATION-START        '# of cycles per 6 hr screen'
502      CALL TIMERREAD'(0,VA0#)
503      IF VA0# < DELAYS*I GOTO 502      'DELAYS=no. of interupts for cycle freq.'
504        LOCATE 2,65:PRINT I:LOCATE 3,65:PRINT VA0#/DELAYS
505        X=START+I+1                  'Increment X variable for graph'
506'
510'   This next line causes K to have the same value for repeated cycles
511'   such that all but the last value is overwritten and only one
512'   value per 15 minutes is saved. (The mean temperature is saved)
515        REP=60*15/CYCLE
516        K=INT(I/REP)
517'
518        XOLD=X            'Resets x axis (time) value.'
520'--Calculate temperatures for sine wave pattern---------------------------
525  HOUR=(DATE-INT(DATE))*24 + I*CYCLE/3600
530  DEG1=MEAN1+SIN(.2618*(HOUR-6))*RANGE1
535  DEG2=MEAN2+SIN(.2618*(HOUR-6))*RANGE2
540  DEG3=MEAN3+SIN(.2618*(HOUR-6))*RANGE3
545  DEG4=MEAN4+SIN(.2618*(HOUR-6))*RANGE4
550  DEG5=MEAN5+SIN(.2618*(HOUR-6))*RANGE5
555  DEG6=MEAN6+SIN(.2618*(HOUR-6))*RANGE6
560'
570'----------Temperature control routines-------------------------------------
572'--Reads temperature, prints temp to screen, draws line, calls control.
574'
576        PUMP=0            'Default pump setting is OFF'
```

```
578       CALL ANREAD'("RESVR",RESVR,13)
580       RESTEMP(K)=RESVR:LOCATE 4,65:PRINT USING"###.#";RESVR
582       LINE(XOLD,RESVROLD)-(X,RESVR),5
584       RESVROLD=RESVR
585'---Chamber 1-------------------------------------------------------------
590       IF TEST1=1 AND MEAN1>=100 THEN GOSUB 2100: GOTO 650
591       IF TEST1=0 AND MEAN1>=100 THEN GOTO 650
592       COLOR 14
594       CALL ANREAD'("CHAMB_1",TEMP1,13)
596       TEMP(1,K)=TEMP(1,K)+TEMP1/REP
598       LOCATE 3,7:PRINT USING"###.##";TEMP1;
600       LINE (XOLD,Y1OLD)-(X,TEMP1)
602       Y1OLD=TEMP1
618       IF TEMP1 >= DEG1+.25 THEN GOSUB 1120: GOTO 650
620       IF TEMP1 > DEG1-1.25 AND TEMP1 < DEG1+.25 THEN GOSUB 1155: GOTO 650
622       IF TEMP1 <= DEG1-1.25 THEN GOSUB 1190: GOTO 650
640'
645'---Chamber 2-------------------------------------------------------------
650       IF TEST2=1 AND MEAN2>=100 THEN GOSUB 2200: GOTO 710
651       IF TEST2=0 AND MEAN2>=100 THEN GOTO 710
652       COLOR 11
654       CALL ANREAD'("CHAMB_2",TEMP2,13)
656       TEMP(2,K)=TEMP(2,K)+TEMP2/REP
658       LOCATE 3,16:PRINT USING"###.##";TEMP2;
660       LINE (XOLD,Y2OLD)-(X,TEMP2)
662       Y2OLD=TEMP2
678       IF TEMP2 >= DEG2+.25 THEN GOSUB 1235: GOTO 710
680       IF TEMP2 > DEG2-1.25 AND TEMP2 < DEG2+.25 THEN GOSUB 1270: GOTO 710
682       IF TEMP2 <= DEG2-1.25 THEN GOSUB 1305: GOTO 710
700'
705'---Chamber 3-------------------------------------------------------------
710       IF TEST3=1 AND MEAN3>=100 THEN GOSUB 2300: GOTO 770
711       IF TEST3=0 AND MEAN3>=100 THEN GOTO 770
712       COLOR 9
714       CALL ANREAD'("CHAMB_3",TEMP3,13)
716       TEMP(3,K)=TEMP(3,K)+TEMP3/REP
718       LOCATE 3,25:PRINT USING"###.##";TEMP3;
720       LINE (XOLD,Y3OLD)-(X,TEMP3)
722       Y3OLD=TEMP3
738       IF TEMP3 >= DEG3+.25 THEN GOSUB 1350: GOTO 770
740       IF TEMP3 > DEG3-1.25 AND TEMP3 < DEG3+.25 THEN GOSUB 1385: GOTO 770
742       IF TEMP3 <= DEG3-1.25 THEN GOSUB 1420: GOTO 770
760'
765'---Chamber 4-------------------------------------------------------------
770       IF TEST4=1 AND MEAN4>=100 THEN GOSUB 2400: GOTO 830
771       IF TEST4=0 AND MEAN4>=100 THEN GOTO 830
772       COLOR 10
774       CALL ANREAD'("CHAMB_4",TEMP4,13)
776       TEMP(4,K)=TEMP(4,K)+TEMP4/REP
778       LOCATE 3,34:PRINT USING"###.##";TEMP4;
780       LINE (XOLD,Y4OLD)-(X,TEMP4)
```

```
782         Y4OLD=TEMP4
798         IF TEMP4 >= DEG4+.25 THEN GOSUB 1465: GOTO 830
800         IF TEMP4 > DEG4-1.25 AND TEMP4 < DEG4+.25 THEN GOSUB 1500: GOTO 830
802         IF TEMP4 <= DEG4-1.25 THEN GOSUB 1535: GOTO 830
820'
825'---Chamber 5-----------------------------------------------------------------
830         IF TEST5=1 AND MEAN5>=100 THEN GOSUB 2500: GOTO 890
831         IF TEST5=0 AND MEAN5>=100 THEN GOTO 890
832         COLOR 12
834         CALL ANREAD'("CHAMB_5",TEMP5,13)
836         TEMP(5,K)=TEMP(5,K)+TEMP5/REP
838         LOCATE 3,43:PRINT USING"###.##";TEMP5;
840         LINE (XOLD,Y5OLD)-(X,TEMP5)
842         Y5OLD=TEMP5
858         IF TEMP5 >= DEG5+.25 THEN GOSUB 1580: GOTO 890
860         IF TEMP5 > DEG5-1.25 AND TEMP5 < DEG5+.25 THEN GOSUB 1615: GOTO 890
862         IF TEMP5 <= DEG5-1.25 THEN GOSUB 1650: GOTO 890
880'
885'---Chamber 6-----------------------------------------------------------------
890         IF TEST6=1 AND MEAN6>=100 THEN GOSUB 2600: GOTO 990
891         IF TEST6=0 AND MEAN6>=100 THEN GOTO 990
892         COLOR 13
894         CALL ANREAD'("CHAMB_6",TEMP6,13)
896         TEMP(6,K)=TEMP(6,K)+TEMP6/REP
898         LOCATE 3,52:PRINT USING"###.##";TEMP6;
900         LINE (XOLD,Y6OLD)-(X,TEMP6)
902         Y6OLD=TEMP6
918         IF TEMP6 >= DEG6+.25 THEN GOSUB 1695: GOTO 990
920         IF TEMP6 > DEG6-1.25 AND TEMP6 < DEG6+.25 THEN GOSUB 1730: GOTO 990
922         IF TEMP6 <= DEG6-1.25 THEN GOSUB 1765: GOTO 990
985'-----------------------------------------------------------------------------
990         IF PUMP => 1 GOTO 1010          'Check pump setting'
995         CALL DIGWRITE'("PUMP",VZERO)
1000'
1010        COLOR 15
1015        IF INKEY$="I" GOTO 3000
1026'-----------------------------------------------------------------------------
1030    NEXT I
1032'---Restart timer for new 6 hour cycle
1035    CALL TIMERSTART'(0)
1040    GOSUB 1820          'Dump data to buffer before start of next 6 hour cycle.'
1045    CLOSE #2            'Closes output file in order to dump buffer to disk.'
1050    I=1                 'Reset counter for start of next 6 hour cycle.'
1055    DATE=DATE+0.25*(DURATION-START)*CYCLE/21600    'Add 1/4 day to the date.'
1060    DAY=INT(DATE)
1065    ERASE TEMP, RESTEMP    'Erase arrays for redeclaration at start of new cycle.'
1070    START=0             'Starting position for new screen'
1075  NEXT J                'Begin next 6 hour cycle.'
1076  J=1                   'Reset counter for start of next 24 hour cycle.'
1077  GOTO 345              'Return to start of 24 hour cycle.'
1080'
```

```
1090'--End timed temperature control loop-----------------------------------
1100'
1115'--Temperature controls for chamber 1-----------------------------------
1120  CALL DIGWRITE'("H1",VZERO)
1125  LOCATE 5,7:PRINT"      ";
1130  CALL DIGWRITE'("C1",VONE)
1132  CALL DIGWRITE'("PUMP",VONE)
1133  PUMP=PUMP+1
1135  LOCATE 6,7:PRINT"   ON  ";
1140'  HEAT(1,I)=0:COOL(1,I)=1
1145  RETURN
1150'
1155  CALL DIGWRITE'("H1",VZERO)
1160  LOCATE 5,7:PRINT"      ";
1165  CALL DIGWRITE'("C1",VZERO)
1170  LOCATE 6,7:PRINT"      ";
1175'  HEAT(1,I)=0:COOL(1,I)=0
1180  RETURN
1185'
1190  CALL DIGWRITE'("H1",VONE)
1195  LOCATE 5,7:PRINT"   ON  ";
1200  CALL DIGWRITE'("C1",VZERO)
1205  LOCATE 6,7:PRINT"      ";
1210'  HEAT(1,I)=1:COOL(1,I)=0
1215  RETURN
1220'-----------------------------------------------------------------------
1225'
1230'--Temperature controls for chamber 2-----------------------------------
1235  CALL DIGWRITE'("H2",VZERO)
1240  LOCATE 5,16:PRINT"      ";
1245  CALL DIGWRITE'("C2",VONE)
1247  CALL DIGWRITE'("PUMP",VONE)
1248  PUMP=PUMP+1
1250  LOCATE 6,16:PRINT"   ON  ";
1255'  HEAT(2,I)=0:COOL(2,I)=1
1260  RETURN
1265'
1270  CALL DIGWRITE'("H2",VZERO)
1275  LOCATE 5,16:PRINT"      ";
1280  CALL DIGWRITE'("C2",VZERO)
1285  LOCATE 6,16:PRINT"      ";
1290'  HEAT(2,I)=0:COOL(2,I)=0
1295  RETURN
1300'
1305  CALL DIGWRITE'("H2",VONE)
1310  LOCATE 5,16:PRINT"   ON  ";
1315  CALL DIGWRITE'("C2",VZERO)
1320  LOCATE 6,16:PRINT"      ";
1325'  HEAT(2,I)=1:COOL(2,I)=0
1330  RETURN
1335'-----------------------------------------------------------------------
```

```
1340'
1345'--Temperature controls for chamber 3-----------------------------------
1350  CALL DIGWRITE'("H3",VZERO)
1355  LOCATE 5,25:PRINT"      ";
1360  CALL DIGWRITE'("C3",VONE)
1362  CALL DIGWRITE'("PUMP",VONE)
1363  PUMP=PUMP+1
1365  LOCATE 6,25:PRINT"  ON  ";
1370'  HEAT(3,I)=0:COOL(3,I)=1
1375  RETURN
1380'
1385  CALL DIGWRITE'("H3",VZERO)
1390  LOCATE 5,25:PRINT"      ";
1395  CALL DIGWRITE'("C3",VZERO)
1400  LOCATE 6,25:PRINT"      ";
1405'  HEAT(3,I)=0:COOL(3,I)=0
1410  RETURN
1415'
1420  CALL DIGWRITE'("H3",VONE)
1425  LOCATE 5,25:PRINT"  ON  ";
1430  CALL DIGWRITE'("C3",VZERO)
1435  LOCATE 6,25:PRINT"      ";
1440'  HEAT(3,I)=1:COOL(3,I)=0
1445  RETURN
1450'-----------------------------------------------------------------------
1455'
1460'--Temperature controls for chamber 4-----------------------------------
1465  CALL DIGWRITE'("H4",VZERO)
1470  LOCATE 5,34:PRINT"      ";
1475  CALL DIGWRITE'("C4",VONE)
1477  CALL DIGWRITE'("PUMP",VONE)
1478  PUMP=PUMP+1
1480  LOCATE 6,34:PRINT"  ON  ";
1485'  HEAT(4,I)=0:COOL(4,I)=1
1490  RETURN
1495'
1500  CALL DIGWRITE'("H4",VZERO)
1505  LOCATE 5,34:PRINT"      ";
1510  CALL DIGWRITE'("C4",VZERO)
1515  LOCATE 6,34:PRINT"      ";
1520'  HEAT(4,I)=0:COOL(4,I)=0
1525  RETURN
1530'
1535  CALL DIGWRITE'("H4",VONE)
1540  LOCATE 5,34:PRINT"  ON  ";
1545  CALL DIGWRITE'("C4",VZERO)
1550  LOCATE 6,34:PRINT"      ";
1555'  HEAT(4,I)=1:COOL(4,I)=0
1560  RETURN
1565'-----------------------------------------------------------------------
1570'
```

```
1575'--Temperature controls for chamber 5-----------------------------------
1580  CALL DIGWRITE'("H5",VZERO)
1585  LOCATE 5,43:PRINT"      ";
1590  CALL DIGWRITE'("C5",VONE)
1592  CALL DIGWRITE'("PUMP",VONE)
1593  PUMP=PUMP+1
1595  LOCATE 6,43:PRINT"  ON  ";
1600'  HEAT(5,I)=0:COOL(5,I)=1
1605  RETURN
1610'
1615  CALL DIGWRITE'("H5",VZERO)
1620  LOCATE 5,43:PRINT"      ";
1625  CALL DIGWRITE'("C5",VZERO)
1630  LOCATE 6,43:PRINT"      ";
1635'  HEAT(5,I)=0:COOL(5,I)=0
1640  RETURN
1645'
1650  CALL DIGWRITE'("H5",VONE)
1655  LOCATE 5,43:PRINT"  ON  ";
1660  CALL DIGWRITE'("C5",VZERO)
1665  LOCATE 6,43:PRINT"      ";
1670'  HEAT(5,I)=1:COOL(5,I)=0
1675  RETURN
1680'-----------------------------------------------------------------------
1685'
1690'--Temperature controls for chamber 6-----------------------------------
1695  CALL DIGWRITE'("H6",VZERO)
1700  LOCATE 5,52:PRINT"      ";
1705  CALL DIGWRITE'("C6",VONE)
1707  CALL DIGWRITE'("PUMP",VONE)
1708  PUMP=PUMP+1
1710  LOCATE 6,52:PRINT"  ON  ";
1715'  HEAT(6,I)=0:COOL(6,I)=1
1720  RETURN
1725'
1730  CALL DIGWRITE'("H6",VZERO)
1735  LOCATE 5,52:PRINT"      ";
1740  CALL DIGWRITE'("C6",VZERO)
1745  LOCATE 6,52:PRINT"      ";
1750'  HEAT(6,I)=0:COOL(6,I)=0
1755  RETURN
1760'
1765  CALL DIGWRITE'("H6",VONE)
1770  LOCATE 5,52:PRINT"  ON  ";
1775  CALL DIGWRITE'("C6",VZERO)
1780  LOCATE 6,52:PRINT"      ";
1785'  HEAT(6,I)=1:COOL(6,I)=0
1790  RETURN
1810'--Write temperature arrays to disk at end of each 6 hour loop----------
1820  LOCATE 20,20:PRINT "END OF 6 HOUR LOOP; DUMPING"
1825  OPEN FILEOUT$ FOR APPEND AS #2
```

```
1830 L=1
1840 FOR L=1 TO K-1
1850   PRINT #2,USING"###.### ";DATE+L*REP*CYCLE/86400;
1855   PRINT #2,USING"###.##
";RESTEMP(L);TEMP(1,L);TEMP(2,L);TEMP(3,L);TEMP(4,L);TEMP(5,L);TEMP(6,L)
1860 NEXT L
1870 RETURN
1880'-------------------------------------------------------------------------
2100'-----Turn off chamber #1-------------------------------------------------
2105 CALL DIGWRITE'("H1",VZERO)
2110 LOCATE 5,7:PRINT"      ";
2115 CALL DIGWRITE'("C1",VZERO)
2120 LOCATE 6,7:PRINT"      ";
2125 TEST1=0
2130 RETURN
2135'
2200'----Turn off chamber #2-------------------------------------------------
2205 CALL DIGWRITE'("H2",VZERO)
2210 LOCATE 5,16:PRINT"      ";
2215 CALL DIGWRITE'("C2",VZERO)
2220 LOCATE 6,16:PRINT"      ";
2225 TEST2=0
2230 RETURN
2235'
2300'----Turn off chamber #3-------------------------------------------------
2305 CALL DIGWRITE'("H3",VZERO)
2310 LOCATE 5,25:PRINT"      ";
2315 CALL DIGWRITE'("C3",VZERO)
2320 LOCATE 6,25:PRINT"      ";
2325 TEST3=0
2330 RETURN
2335'
2400'----Turn off chamber #4-------------------------------------------------
2405 CALL DIGWRITE'("H4",VZERO)
2410 LOCATE 5,34:PRINT"      ";
2415 CALL DIGWRITE'("C4",VZERO)
2420 LOCATE 6,34:PRINT"      ";
2425 TEST4=0
2430 RETURN
2435'
2500'----Turn off chamber #5-------------------------------------------------
2505 CALL DIGWRITE'("H5",VZERO)
2510 LOCATE 5,43:PRINT"      ";
2515 CALL DIGWRITE'("C5",VZERO)
2520 LOCATE 6,43:PRINT"      ";
2525 TEST5=0
2530 RETURN
2535'
2600'----Turn off chamber #6-------------------------------------------------
2605 CALL DIGWRITE'("H6",VZERO)
2610 LOCATE 5,52:PRINT"      ";
```

```
2615 CALL DIGWRITE'("C6",VZERO)
2620 LOCATE 6,52:PRINT"      ";
2625 TEST6=0
2630 RETURN
2635'
2999'--Write temperature arrays to disk if program interrupted----------
3000 LOCATE 20,20:PRINT "INTERRUPT SUB; DUMPING"
3015 OPEN FILEOUT$ FOR APPEND AS #2
3020 L=1
3030 FOR L=1 TO K-1
3040   PRINT #2,USING"###.### ";DATE+L*REP*CYCLE/86400;
3045   PRINT #2,USING"###.##
";RESTEMP(L);TEMP(1,L);TEMP(2,L);TEMP(3,L);TEMP(4,L);TEMP(5,L);TEMP(6,L)
3050 NEXT L
3060'------------------------------------------------------------------------
3070'
3080'--Turn off interrupts and turn off all temperature controls----------
3090 CALL INTOFF
3100'
3110 CALL DIGWRITE'("C1",VZERO)
3120 CALL DIGWRITE'("H1",VZERO)
3130 CALL DIGWRITE'("C2",VZERO)
3140 CALL DIGWRITE'("H2",VZERO)
3150 CALL DIGWRITE'("C3",VZERO)
3160 CALL DIGWRITE'("H3",VZERO)
3170 CALL DIGWRITE'("C4",VZERO)
3180 CALL DIGWRITE'("H4",VZERO)
3190 CALL DIGWRITE'("C5",VZERO)
3200 CALL DIGWRITE'("H5",VZERO)
3210 CALL DIGWRITE'("C6",VZERO)
3220 CALL DIGWRITE'("H6",VZERO)
3225 CALL DIGWRITE'("PUMP",VZERO)
3230 END
```

Chapter 3

Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature-time requirements of prediapause development.

Eggs of the gypsy moth (*Lymantria dispar* (L.) are laid in midsummer. After a period of morphological development, eggs enter a diapause state for the winter and resume morphological development in the spring. Eggs hatch in May or June (Doane and McManus 1981). Although the ontogeny of gypsy moth eggs is commonly described as comprising the three phases of prediapause morphological development, diapause, and postdiapause morphological development, the precise relationship between, or uniqueness of, the phases is unclear. Thus, despite extensive research into the effects of temperature on gypsy moth egg phenology, no robust model of the process exists. Lyons and Lysyk (1988) claim this may be due mainly to an inability to distinguish the phases of egg ontogeny. Attempts to predict egg hatch with models of postdiapause phenology rely on untested assumptions regarding time of initiation of the postdiapause phase (Johnson et al. 1983, Lyons and Lysyk 1988). These assumptions can lead to actual egg hatch preceding simulated egg hatch by as much as 25 d (S. L. Smith, USDA Forest Service, Redding, Calif., personal communication).

Almost without exception, researchers have interpreted the effects of temperature on the diapause process by measuring time to hatch, distribution of hatch, and hatch success (e.g., Masaki 1956, Maksimovic 1958, Pantyukhov 1964, Lyons and Lysyk 1988, Tauber et al. 1990). Egg hatch is, however, a developmental phenomenon physiologically and temporally quite distinct from diapause. Typically, eggs have been collected from the field after experiencing ambient conditions of unknown character. This has resulted in untested assumptions regarding the state of ontogeny of the eggs at the onset of an experiment. Inability to distinguish the diapause state from states of pre- or postdiapause morphological development has prevented researchers from knowing if temperature

treatments were applied to, or had affected, a diapause process or a morphological process or both.

The diapause state has been characterized by arrested morphological development, reduced rates of respiration (Harvey 1962, Zaslavski 1988) and protein synthesis (Hayes et al. 1972, Venkatesh and Chippendale 1986), and increased titre of polyhydric alcohols (Nordin et al. 1984). In individual species, diapause may be characterized by either increased or decreased levels of a hormone or neurohormone (Chippendale 1988). Biochemical procedures used to distinguish developmental phases have the disadvantage of being destructive, and the precise time of phase change cannot be observed. In addition, such destructive procedures preclude following a single individual through all phases. Measurements of respiration rate as an indication of the diapause state have required the use of several hundreds of individuals per sample (Pantyukhov 1964, Bell 1989), effectively obscuring the variability of the population in duration of either the diapause or prediapause phases. Measurement techniques required several hours at constant 25°C, creating additional problems when estimating the effects of lower temperatures.

The objectives of this study were to (1) develop and test a non-destructive technique for distinguishing developmental phases in individual insect eggs; (2) describe the temperature-dependent prediapause developmental rate of gypsy moth eggs; (3) describe variability in prediapause developmental rate of a population of gypsy moth eggs; and (4) discuss the application of the technique to test current hypotheses regarding diapause termination in gypsy moth.

Materials and Methods

Gypsy moth pupae were obtained from a colony at the Insect Reproduction Laboratory, USDA/ARS, Beltsville, MD. The colony was originally derived from a New

Jersey population field-collected in 1967 and is widely used for laboratory and field studies (Bell 1989).

Pupae were placed in a 25°C constant temperature chamber. Eclosed adults were allowed to mate, and egg masses were collected within 24 h of the onset of oviposition. Egg masses were dehaired by hand-rubbing them with paper towels in a fume hood with an air velocity of 3.6 m^3 x min^{-1}. Individual eggs were placed in 1-ml glass autosampler vials (Fisher Scientific, Pittsburgh, PA, 03-340-5A,) and reared under constant temperature (4, 15, 20, 25, 30, 35, or 38°C) and a 12:12 (L:D) photoperiod. All treatments began within 24 h of oviposition.

Respiration rates for all experiments were determined after sealing the vials with a rubber septum (Fisher 03-340-13A). Four control vials per temperature treatment were sealed without an egg to obtain estimates of ambient CO_2 concentration. After approximately 24 h a 250 *u*l gas sample was withdrawn from each vial using a gas-tight syringe (Supelco, Belefonte, PA, 2-0739) and injected into a closed-system infrared gas analyzer (IRGA) (Li-Cor Inc., Lincoln, NB, LI-6200). The CO_2 concentration within each vial was calculated as the product of the IRGA response to CO_2 and a dilution factor (volume of IRGA/volume of syringe: 52.1 ml/250 *u*l). The volume of CO_2 within each vial was calculated as the product of vial CO_2 concentration and vial volume. Respiration rate (*u*l CO_2/24 h) of each egg was estimated by subtracting the mean CO_2 volume of the four control vials (from the same temperature chamber) from the calculated CO_2 volume within the egg-containing vial and dividing by the proportion of a 24-h period during which the vial was sealed.

Experiment 1: Effect of Temperature and Age on Respiration Rate. Eggs of similar physiological age were used to determine the effect of temperature on the respiration rates of individual eggs in the prediapause phase. Newly laid eggs were

stored at constant 25°C. At approximately 48-h intervals for 40 d, 30 eggs were randomly selected from a single egg mass and placed in individual vials. Five eggs were placed in each temperature chamber. Eggs were allowed to acclimatize for approximately 4 h before vials were sealed and CO_2 measurements were taken as described above. Eggs were discarded after CO_2 was measured. Measurements were not made at 20°C on days 36 and 40 and at 15°C on day 40 because of equipment failure.

Data were tested for normality by the Shapiro-Wilk *W* statistic (SAS Institute 1985). The ability of the IRGA to detect a greater-than-ambient CO_2 concentration due to the presence of an egg was tested by the Wilcoxon rank sum test (vials with eggs versus control vials) for each temperature x measurement date combination. For each measurement date, the effect of temperature on respiration rate was determined by the Kruskal-Wallis test, and treatments were separated by the Wilcoxon rank sum test where appropriate (Hollander and Wolfe 1973).

Thermal response of respiration rate was estimated by linear regression of rate on temperature for each measurement date using the Theil-Sen method of slope estimation and the Hodges-Lehmann method of y intercept estimation (Hollander and Wolfe 1973). The effect of age on the thermal response of respiration rate was examined by comparing estimated slopes and their confidence intervals (Hollander and Wolfe 1973) of the respiration rate-temperature function from each measurement date.

Experiment 2: Effects of temperature on prediapause duration. The effect of temperature on prediapause duration was determined by randomly allocating five eggs from each of four egg masses to each temperature treatment. Respiration rates were determined for all eggs at approximately 48-h intervals for 40 days (33 d for 20°C). After respiration rate was measured, vials were decapped and eggs were returned to their respective chambers. The transition to the diapause state may occur over a period of

several days (Bell 1989); it is difficult to say when the transition is complete. For this reason, diapause entry (completion of the prediapause phase) for each egg was arbitrarily chosen to be the point in time when its respiration rate declined to 50% of its maximum.

Constant temperatures of 4 and 38°C were lethal to all eggs within 8 days. Duration of prediapause under these extreme temperatures was determined by the method of Regniere (1987). Eggs from a single mass were temporarily transferred from 25°C to the extreme temperature. The portion of development that occurred at 25°C was calculated as the product of median developmental rate (duration^{-1}) at 25°C (determined from eggs that experienced constant 25°C) and time spent at 25°C. Developmental rate at the extreme temperature was calculated as the portion of development that did not occur at 25°C divided by the time spent at the extreme temperature. Because eggs from only a single mass were used, estimates of variability among egg masses are not available for these temperatures. Eggs within the 15 and 20°C treatments spent 6 days at 25°C because of equipment malfunction, and prediapause duration under these temperatures was also calculated by the method of Regniere (1987).

Data were tested for normality by the Shapiro-Wilk *W* statistic (SAS Institute 1985). Within each temperature x measurement date combination, the effects of egg mass were examined by one-way analysis of variance (ANOVA) of the respiration rate ranks (equivalent to the Kruskal-Wallis test) (SAS Institute 1985). The effects of egg mass and temperature on prediapause duration were examined by a two-way ANOVA of duration ranks (equivalent to the Freidman test) (SAS Institute 1985).

To describe the temperature-dependent rate of prediapause development, equation 6 of Logan et al. (1976),

$$R_D(T) = \psi(e^{\rho T} - e^{\rho T_M - \tau}) \qquad [2]$$

was fit to the median rate (inverse of prediapause duration), where $R_D(T)$ is developmental rate at T°C above the 4°C base temperature, T_M is the lethal, maximum temperature in degrees above the base temperature, ψ is the developmental rate at base temperature, ρ is the rate of increase to optimum temperature, $\tau = {(T_M - T)}/{\Delta_T}$ and Δ_T is the width of the high level boundary layer (i.e. the temperature span over which the developmental rate function changes its behavior) (Logan et al. 1976)

Variability of the population was described by fitting a three parameter cumulative Weibull function,

$$F(x) = 1 - e^{-\left\{(x-\gamma)/\beta\right\}^{\alpha}},$$

to the rates within each temperature treatment. F(x) is the cumulative probability of developmental rate x; and α, β, and γ are estimated parameters (Law and Kelton 1982). Normalized rates were calculated by dividing each rate by the median rate of the same treatment. A single, temperature-independent estimate of variability was made by fitting a three-parameter cumulative Weibull function to the pooled normalized rates (Sharpe et al. 1977, Wagner et al. 1984). Curves were fit by the least squares method of Powell (1964).

Results

Many subsets of the data failed to satisfy the test for normality ($P<0.05$). Therefore, nonparametric statistical procedures were judged to be more reliable than their parametric counterparts. All combinations of positive or negative skewness with lepto- or playtkurtosis were observed. The manner of departure from normality was inconsistent and precluded any conclusions regarding the distributions.

Experiment 1: Effect of Temperature and Age on Respiration Rate. The IRGA responses to CO_2 concentration were significantly greater from vials with eggs than control vials under all temperature conditions for all measurement dates. Even at 4°C,

responses were significantly greater from vials with eggs than control vials immediately following oviposition (day 1: W=29; m,n=5,4; P=0.016) and after respiration had declined to steady, low rates indicative of diapause (day 25: W=30; m,n=5,4; P=0.008). For this reason, the method of measuring respiration rates of individual eggs was judged to be satisfactory.

Temperature had a significant effect (day 36: H=17.00; df=4; P<0.01; day 40: H=12.66; df=3; P<0.01; all other days: $H \geq 16.65$; df=5; P<0.01) on respiration rate among eggs of similar physiological age from time of oviposition through early diapause. Median respiration rate was observed to increase rapidly after day 7 under all temperature conditions except 4°C and to reach a maximum on day 13 (Fig. 3.1). Thereafter, respiration rate declined sharply. Except for an unexplained increase on day 33 at 30°C, respiration rate remained low for the duration of the experiment.

Thermal responses of respiration rate were significant for all measurement dates as indicated by significant (P<0.0001) positive slopes of all regression functions. The magnitude of the response (Fig. 3.2) followed a pattern similar to that of the median respiration rates (Fig. 3.1). A slight increase in thermal response from day 1 to day 7 was followed by a rapid increase until day 11 and a rapid decrease until day 22.

Experiment 2: Effects of Temperature on Prediapause Development.

Respiration rates of eggs reared in constant temperature treatments of 25 and 30°C displayed similar patterns. A short period of gradual increase was followed by a period of rapid increase, then a rapid decline to near initial rates. Thereafter, the respiration rate of each individual remained low for the duration of the experiment, suggesting that diapause had been completely entered (Fig. 3.3).

Individual respiration rates were significantly different (P<0.05) among egg masses

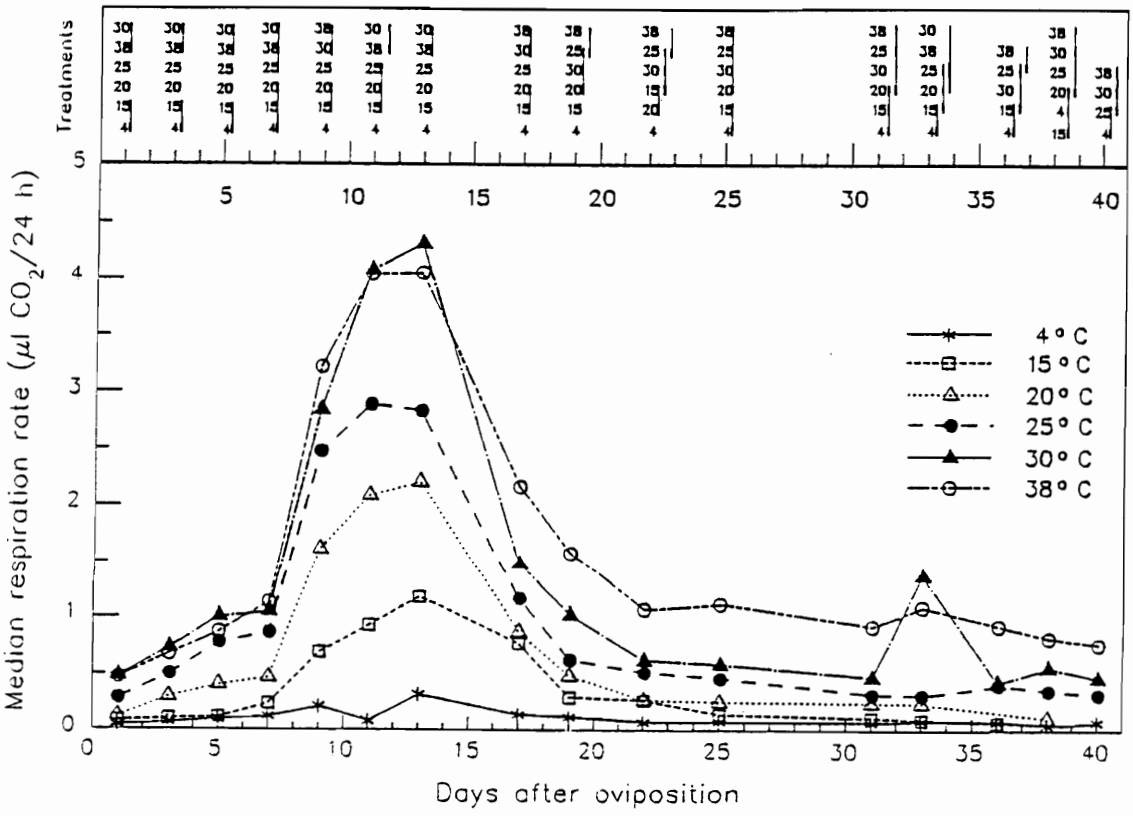

Fig. 3.1. Respiration rates (ul CO_2/24h) of individual gypsy moth eggs of similar physiological age under six temperature conditions for 40 d immediately following oviposition; all eggs reared at 25°C (bottom). Treatments connected by lines had not significantly different median respiration rates; Wilcoxon rank sum test (P=0.05) (top).

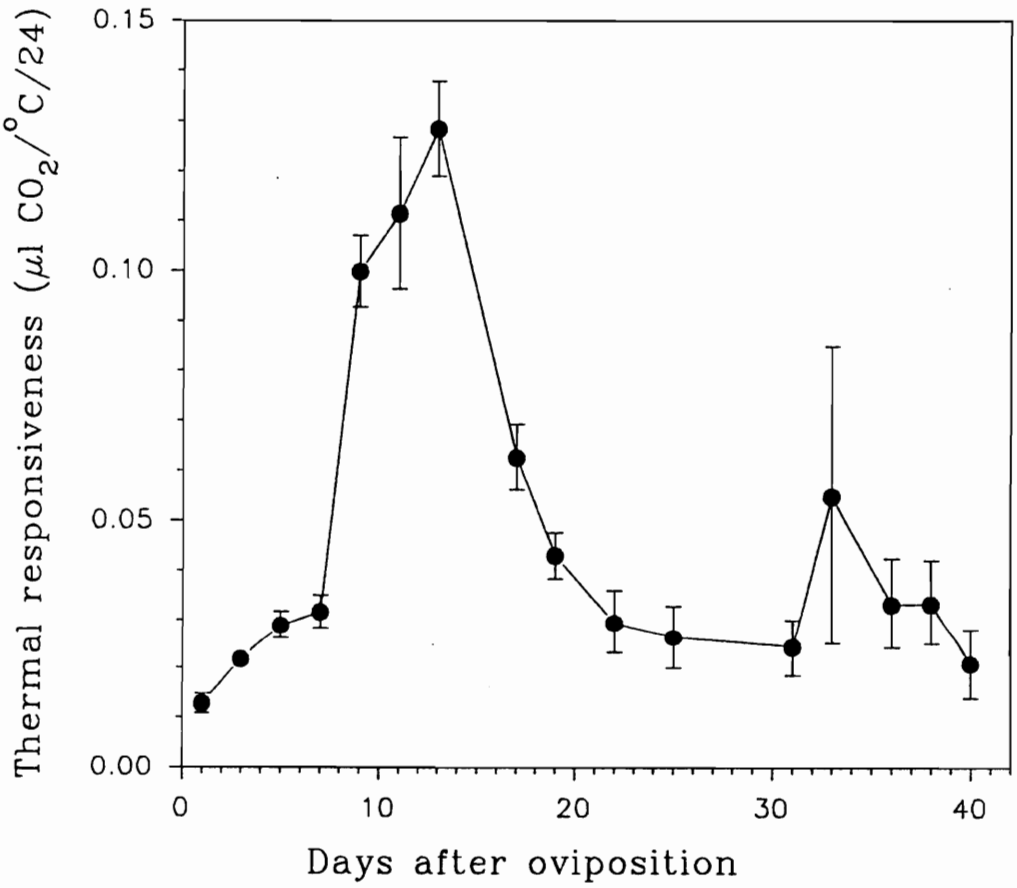

Fig. 3.2. Slope (●) and 95% CI of the estimated linear relationships between respiration rate and temperature (thermal response function) for 40 d following oviposition.

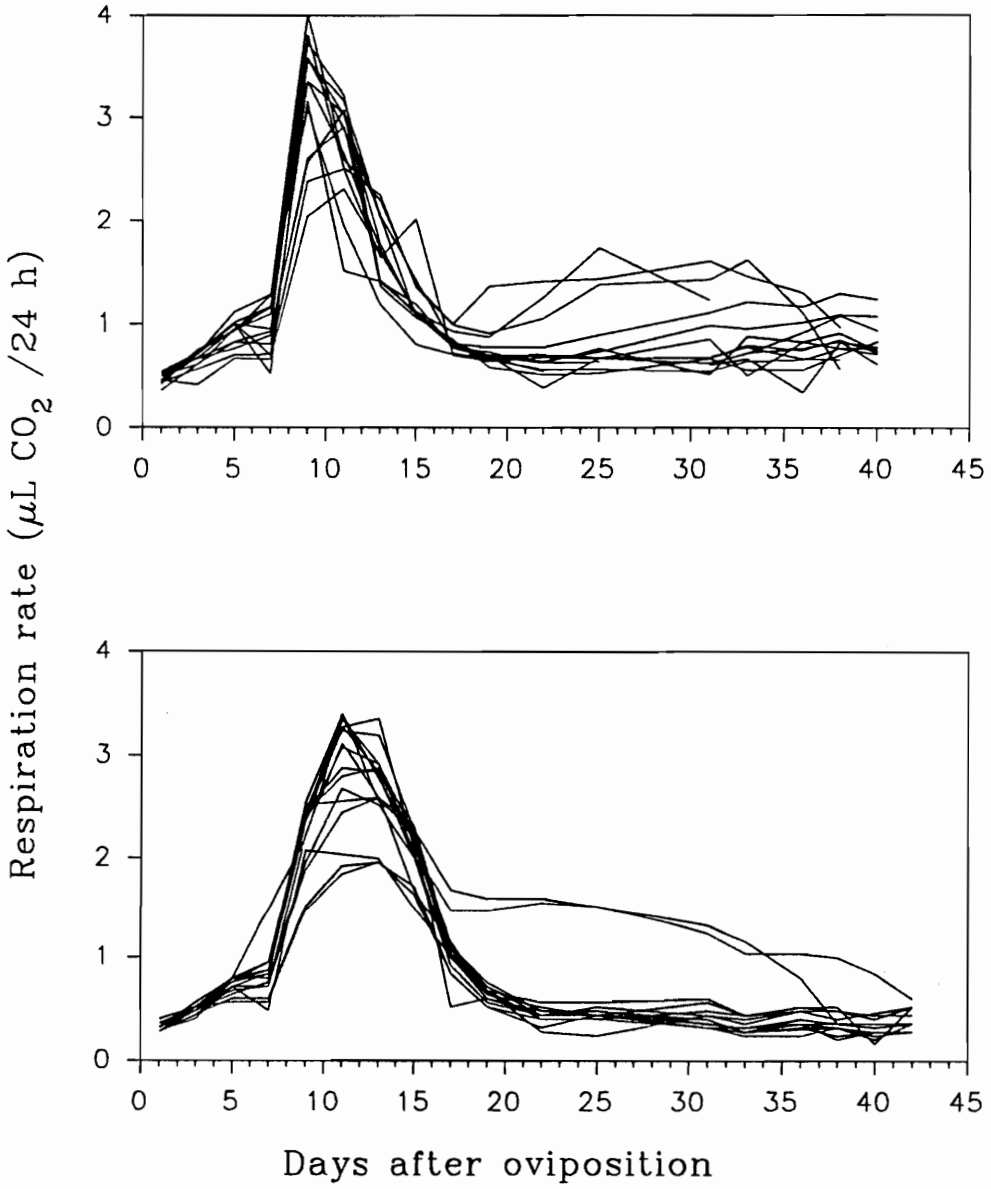

Fig. 3.3. Individual respiration rates (μl CO_2/24h) of 20 gypsy moth eggs under constant temperature regimes of 30 (top) and 25°C (bottom) for 40 d immediately following oviposition.

in approximately 28% of the temperature x measurement date combinations. However,the differences were not consistent across dates or temperature treatments.

Prediapause duration was not significantly different among egg masses (F=1.02; df=3,9; P=0.43), but was significantly different among temperature treatments (F=122.64; df=5,9; P<0.0001). Data from all egg masses were therefore combined to estimate the temperature-dependent prediapause developmental rates and variability of the population in these rates. Time of egg hatch also has been observed to have greater variability within than among egg masses (Lyons and Lysyk 1988).

The relationship between median rate of prediapause development (i.e., inverse of median prediapause duration) and temperature was well described by equation 6 of Logan et al. (1976) (Fig. 3.4). Median developmental rates were 0, 0.021, 0.040, 0.062, 0.074, and 0 d^{-1} in the 4, 15, 20, 25 30, and 38°C treatments respectively. The data suggest a maximum prediapause developmental rate of 0.077 d^{-1} at 31°C. A temperature-independent estimate of population variability in prediapause developmental rate is shown in Fig. 3.5. Parameter estimates for all functions are given in Table 1.

Discussion

A method of distinguishing the phases of insect ontogeny that encompass the diapause state is essential to understanding the effects of environmental factors such as temperature, photoperiod, and humidity on rates of development. Only an adequate understanding will lead to an adequately modelled process. An inability to determine the phase(s) of ontogeny being affected by an experimental treatment has led to several conflicting models of insect phenology that include the diapause phase.

Holtzer et al. (1976) described a conceptual form of a generalized diapause termination model comprising two distinct phases: diapause development and

Table 1. Parameter estimates of functions used to describe the prediapause developmental rate of gypsy moth eggs

Function	ψ	ρ	Δ_T	T_M	β	α	γ	R^2
median rate: $R_D(T) = \psi(e^{\rho T} - e^{\rho T_M - \tau})$								
where $\tau = T_M - T / \Delta_T$								
	.0191	.1455	6.350	33.993				.960
population variability: $F(x) = 1 - e^{-\left\{(x-\gamma)/\beta\right\}^{\alpha}}$								
15°C					41.258	7.950	7.990	.954
20°C					24.315	21.328	1.340	.914
25°C					13.944	31.947	2.411	.962
30°C					11.228	14.036	2.406	.972
temp. indep.					0.522	10.060	0.499	.982

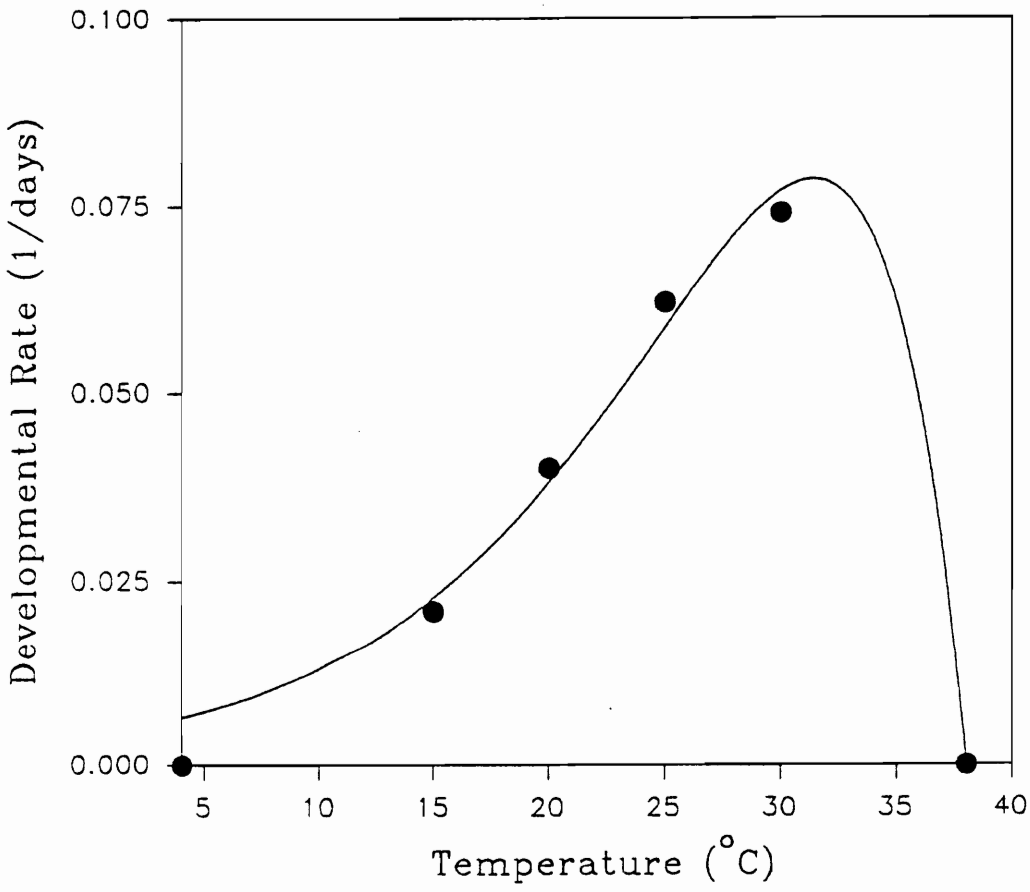

Fig. 3.4. The relationship between observed (●) median developmental rate (R_D) and temperature (T); and the relationship (——) as estimated by: $R_D(T) = \psi(e^{\rho T} - e^{\rho T_M - \tau})$. $\psi = 0.0191$; $\rho = 0.1455$; $\Delta_T = 6.350$; $T_M = 33.993$; $\tau = (T_M - T)/\Delta_T$; $R^2 = .960$

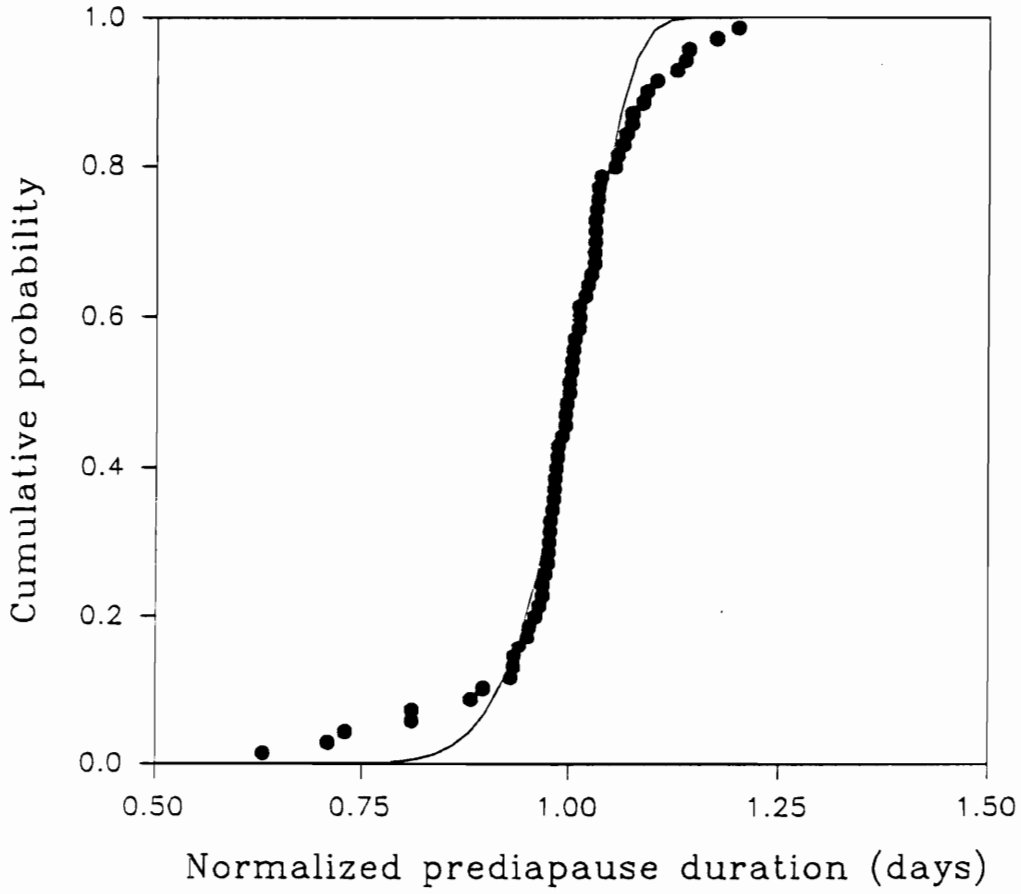

Fig. 3.5. Observed (●) cumulative frequency distribution of relative development times (days) to enter diapause; and the estimated (——) cumulative frequency distribution of relative development times as estimated by: $F(x) = 1 - e^{-\left\{(x-\gamma)/\beta\right\}^{\alpha}}$. $\beta = 0.552$; $\alpha = 10.060$; $\gamma = 0.499$; $R^2 = .982$

morphological development. They hypothesized that each phase is governed by its own temperature-dependent rate function and that the phases are sequential. Logan et al. (1979) used a two-phase model to predict spring emergence of *Heliocoverpa zea* (Boddie). The requirement that one phase be wholly or partly completed before the subsequent phase begins was hypothesized by Moore (1948). On the other hand, Hilbert et al. (1985) have hypothesized a process where diapause development (or a diapause-regulating process) may proceed simultaneously with prediapause development. The diapause-regulating process always occurs; however, the diapause state is manifested only under certain conditions. More recently, Tauber et al. (1990) proposed a dynamic diapause process wherein insects exhibit changes in thermal response as the phase progresses, and where there is no clear transition between diapause and postdiapause states. Regniere (1990) convincingly hypothesizes gradually changing thermal responsiveness during the postdiapause phase of spruce budworm, *Choristoneura fumiferana* (Clemens). However, diapause was only assumed to be completed at the onset of the experiment. These hypotheses arise from experiments that were unable to distinguish diapause and morphological phases.

Tauber et al. (1990) cited the data of Masaki (1956) as evidence of a dynamic diapause process . However, these same data can also be interpreted within the framework of a three-phase process (prediapause, diapause, and postdiapause developments) where the developmental rate within each phase is governed by its own temperature-dependent rate function and the phases are sequential, with a clear transition between each. Pre- and postdiapause phases are characterized by morphological development, and the diapause phase is characterized by arrested morphogenesis and reduced metabolic activity.

Masaki (1956) observed that as the time spent at 5°C increased, incubation time at high temperature required for egg hatch decreased. However, the rate of decrease was not constant; increasing the time at 5°C from 80 to 90 days resulted in a decrease in incubation time from 21 to 17 days, whereas a nearly equal increase in time at 5°C from 145 to 160 days decreased the incubation time from 7 to 6 days (Fig. 3.6). This nonlinear thermal response prompted the suggestion of a dynamic diapause phase (Tauber et al. 1990).

However, by iteratively fitting two linear curves to different subsets of Masaki's (1956) data until the best fit is obtained by both curves (Fig. 3.6), there is evidence of a transition occurring at day 111. If the rate curves for the diapause and postdiapause phases overlap (such as in Fig. 3.7), an extra day at 5°C before day 111 would have a greater effect on reducing incubation time than an extra day after day 111. Premature transfer to 26°C would retard the completion of diapause that is a pre-requisite for the initiation of the postdiapause phase. Extending the cold treatment past day 111 has a lesser effect on incubation requirements because the insect is experiencing cool temperatures during the postdiapause phase. Unpublished data (M. L. McManus, USDA Forest Service, Hamden Conn., personal communication) suggest a phase transition at day 110 during a 4.4°C cold treatment (Fig. 6).

Data presented by Tauber et al. (1990) and interpreted as additional evidence of a dynamic thermal response can also be interpreted within the context of a three-phase sequential model with overlapping rate functions (Table 2; Table 5 of Tauber et al. 1990). The decreasing relative times to hatch that occur with the later transfer dates could be explained by the fact that a later transfer date implies a more advanced diapause phase. With a smaller proportion of the diapause phase still to be completed, transfer to a suboptimum temperature will retard egg hatch by a smaller amount. The increases in

Table 2. Relative times to median hatch of gypsy moth eggs after transfer from out-of-doors to five thermal conditions under two photoperiods[a]

	Ratio of Medians[b]				
Photoperiod (L:D) Transfer date	Temperature, °C				
	10.0	15.6	18.3	21.1	23.9
16:8					
22 Sept.	2.6	2.5	2.4	1.8	1.0
31 Oct.	2.9	2.3	2.0	1.1	1.0
30 Dec.	2.9	1.5	1.3	1.1	1.0
30 Jan.	-	1.5	1.4	1.0	1.0
10:14					
31 Oct.	3.3	2.1	1.6	1.2	1.0
30 Dec.	3.3	1.6	1.4	1.1	1.0
30 Jan.	-	1.8	1.4	1.1	1.0
22 March	-	1.6	1.3	1.1	1.0

[a] From Tauber et al. (1990).
[b] Calculated by dividing median emergence time at each temperature by median emergence time at 23.9°C.

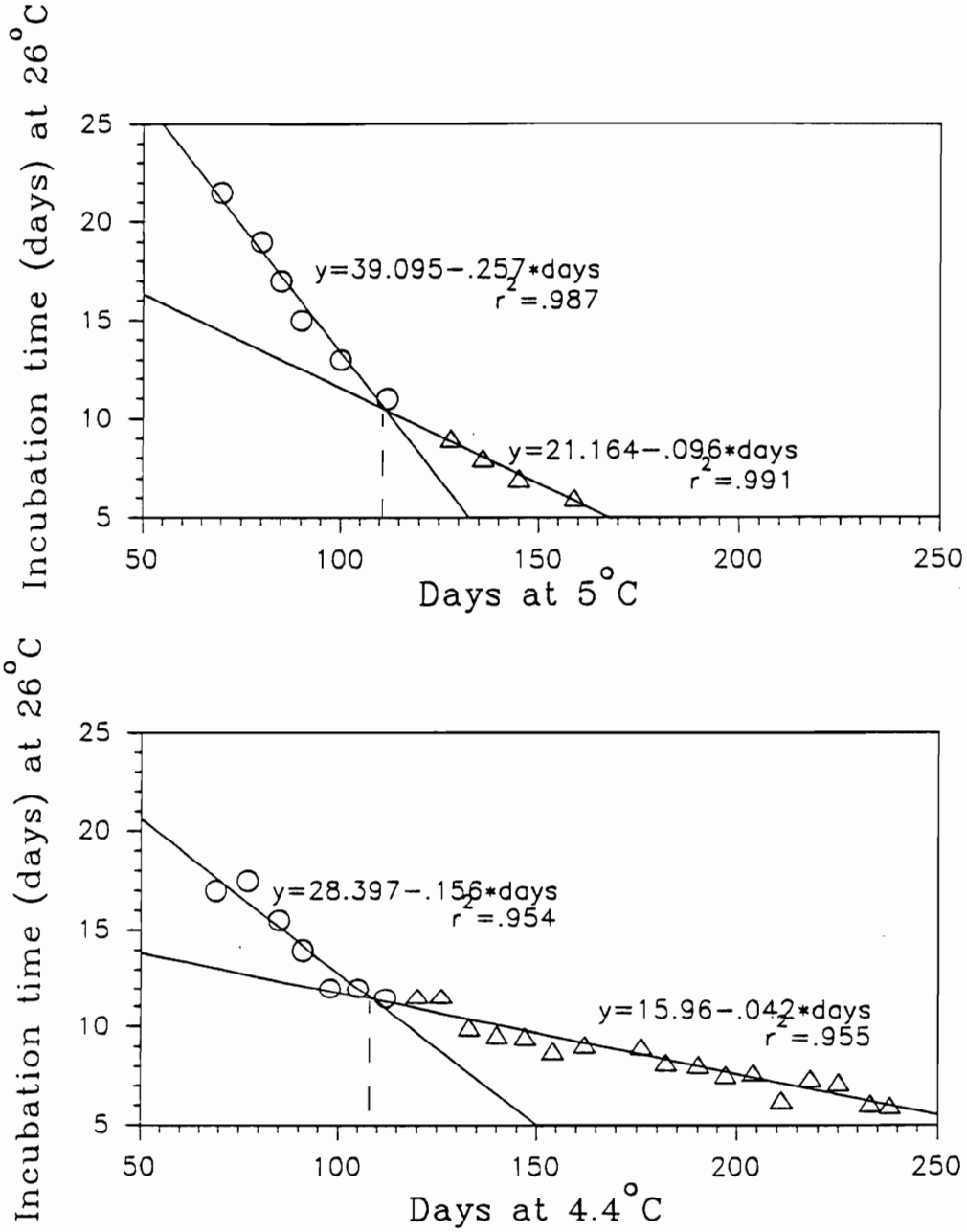

Fig. 3.6. The relationships between observed (●) mean incubation time at 26°C required for hatch of gypsy moth eggs from Hokkaido Island and Yokohama, Japan (top) (from Masaki 1956) and Connecticut (bottom) (from McManus, unpublished data) after exposure to cold and the duration of the cold treatment; and the hypothesized relationship (——) based on a phase transition.

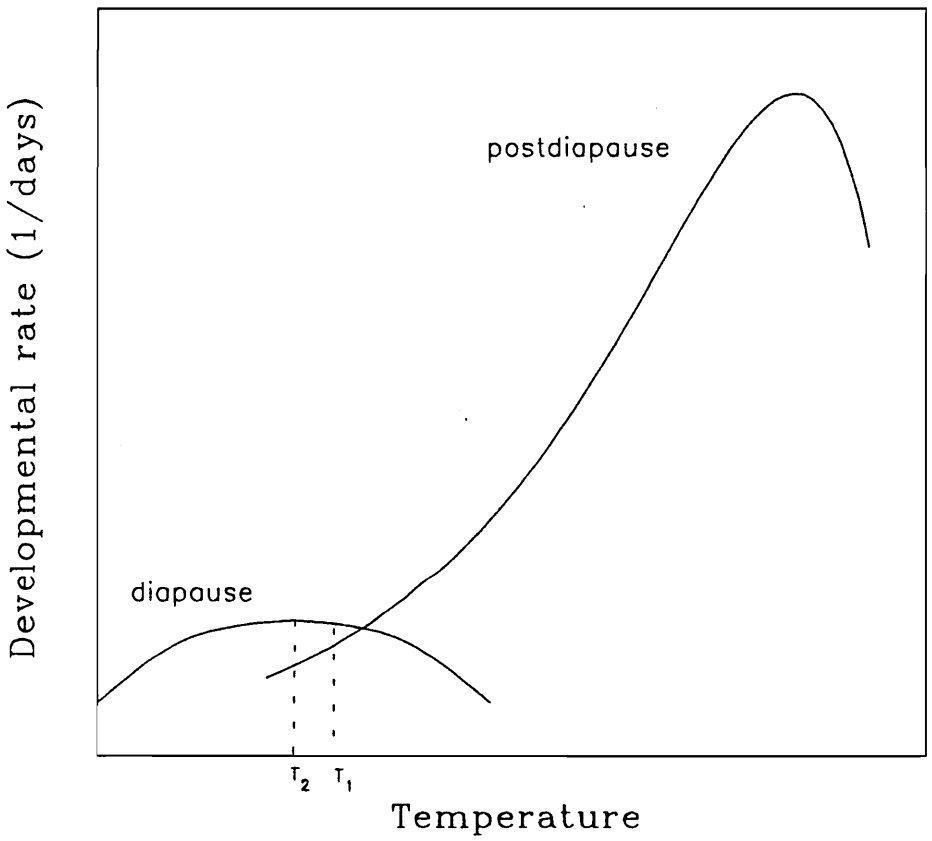

Fig. 3.7. Hypothetical relationships between developmental rates and temperature for diapause and postdiapause developmental processes.

relative times to hatch that occur with decreasing transfer temperature could occur if the lower temperature causes a proportionately greater decrease in the rate of postdiapause development than increase in the rate of diapause development. For example, a decrease in temperature from T_1 to T_2 would decrease the postdiapause developmental rate more than it would increase the diapause rate (Fig. 3.7).

Thus, gypsy moth egg phenology may comprise two or more phases: simultaneous (Hilbert et al. 1985), dynamic (Regniere 1990, Tauber et al. 1990), or sequential (Moore 1948, Holtzer et al. 1976). A means of distinguishing the phases is essential to determining the independence or interaction of the phases, and estimating the effects of environmental factors such as temperature, photoperiod, and humidity on the interactions or on the rates of those phases, or both. A satisfactory model must also account for variability of the population in developmental rate response to the environmental factors.

The technique presented here offers a means of using a physiologically-based variable (CO_2 evolution) to distinguish the phases of egg phenology. Its non-destructive nature allows the interaction or independence of the phases to be examined within a single individual. Its sensitivity will permit observation of phase transition (including diapause termination) under various temperature conditions, thereby providing a method to estimate photoperiod-, humidity-, or temperature-dependent rate functions associated with each phase. Estimation of variability in a population's rate response to governing environmental factors is also possible by examination of individual eggs.

In addition, this technique provides a method of directly investigating the question of diapause depth. Numerous researchers have relied on duration of diapause or emergence time as an indicator of diapause depth (e.g., Tauber et al. 1986, Regniere 1990). It is proposed that the criterion of thermal responsiveness as indicated by respiration rate be used as a measure of diapause depth.

The data presented here show that temperature has a pronounced effect on the rate of prediapause development. Under constant temperature conditions, entry into the diapause phase is signalled by a rapid decline in the respiration rate of individual eggs. In 25 and 30°C conditions, diapause entry may begin 13 and 10 days after oviposition, respectively (Fig. 3.3a, b). Although the diapause phase may be characterized by a reduced respiration rate, it is not simultaneously characterized by a complete absence of thermal sensitivity (Fig. 3.2). The response of respiration rate to temperature remained significantly positive, even while respiration rates were at their lowest. However, thermal sensitivity was seen to be significantly lower in the diapause phase than the prediapause phase.

Addendum

Following publication of this chapter (Gray et al. 1991) the method of estimation of thermal response of respiration rate during prediapause and early diapause was re-examined for the following reason. A change in physiological state (eg. from a nondiapausing to a diapausing state) may affect respiration rates in two ways. Firstly, respiration rates may be universally lowered. Secondly, respiration rates in the diapause state may be less affected by changes in temperature. These two effects must be examined separately.

In this chapter, thermal responsiveness was equated with sensitivity to temperature and was defined as the change in respiration rate per degree change in temperature (μl CO_2/24 h/°C). However, if a change in physiological condition reduces respiration rates at all temperatures by an equivalent proportion, the relative response to a temperature change is identical between the two physiological conditions. Because temperature produced changes of equal magnitude, there is equal thermal sensitivity between the physiological conditions. However, universally lower respiration rates will decrease the

change in respiration rate per degree change in temperature. Thus the pattern of thermal responsiveness shown in Fig. 2 may be due, in part, to changes in respiration rate at all temperatures and not due to changes in sensitivity to temperature caused by changes in physiological condition.

Therefore, an examination of the sensitivity of respiration rate to temperature during egg development is more accurately examined by first removing any uniform changes in respiration rate that may occur due to a change in physiological condition. This can be done by estimating the effect of of temperature on relative respiration rates. Relative respiration rates for each measurment date were calculated by dividing each respiration rate by the mean respiration rate at 25°C on the same day. Thermal responsiveness was then estimated by linear regression of relative rate on temperature for each measurment date as described earlier. Relative respiration rates and residuals from the linear relationship were visually examined for evidence of a nonlinear thermal responsiveness.

There was a significant thermal responsiveness for all measurement dates as indicated by significant ($p < 0.0001$) positive slopes of all regression functions. There was no evidence of a nonlinear relationship. There was no distinct pattern to the estimated values of thermal responsiveness during the 40 days following oviposition (Fig. 3.8). Estimates ranged from 0.0304 $\Delta R/_C$ on day 22 to 0.0654 on day 38, where ΔR is the dimensionless change in relative respiration rate. Overlapping 95% confidence intervals of estimates indicates there was no significant difference among estimates. Therefore, the changes in the relationship between respiration rate and temperature are due to proportionally equal changes in respiration rate over all temperatures and not to changes in sensitivity to temperature. The onset of diapause is indicated by the sudden decline in respiration rate of individual eggs (Fig. 3.3) and causes a reduction of

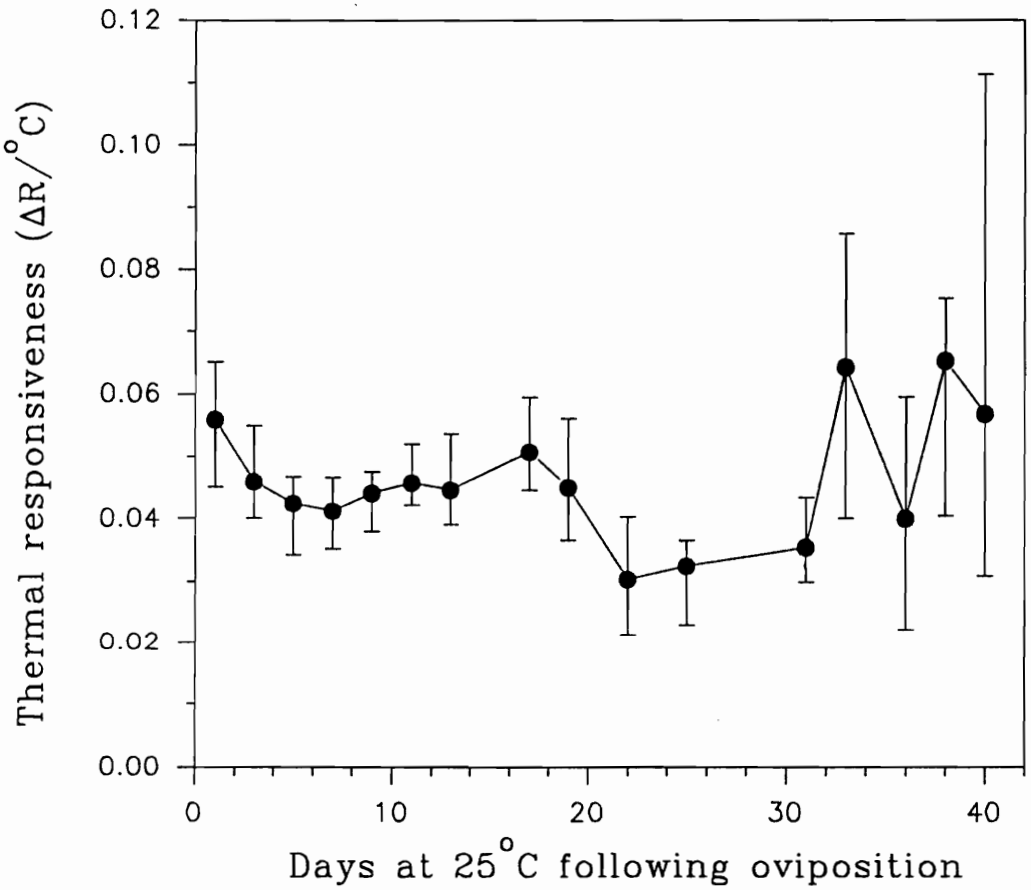

Fig. 3.8. Estimate (●) and 95% CI of the thermal responsiveness for 40 d following oviposition. Values estimated from the linear relationship between respiration rate (expressed as proportion of the rate at 25°C on the same day) and temperature for each measurement day.

respiration rate at all temperatures. Diapause does not, however, render individuals less sensitive to temperature.

References Cited

Bell, R. A. 1989. Respiratory activity during embryonic development in a diapausing and a selected non-diapausing strain of the gypsy moth, *Lymantria dispar* L. Comp. Biochem. and Physiol. 93A: 761-771.

Chippendale, G. M. 1988. Role of proteins in insect diapause, pp. 331-46. In Sehnal, F., A. Zabza, and D. L. Denlinger [eds.], Endocrinological frontiers in physiological insect ecology. Wroctaw Technical University Press, Wroctaw, Poland.

Doane, C. C., and M. L. McManus. 1981. The gypsy moth: research toward integrated pest management. USDA For. Serv. Tech. Bull. 1584.

Harvey, W. R. 1962. Metabolic aspects of insect diapause. Annu. Rev. Entomol. 7: 57-80.

Hayes, D. K., P. S. Reynolds, J. U. McGuire and M. S. Schechter. 1972. Synthesis of macromolecules in diapausing and nondiapausing larvae of European corn borer. J. Econ. Entomol. 65: 676-679.

Hilbert, D. W., J. A. Logan and D. M. Swift. 1985. A unifying hypothesis of temperature effects on egg development and diapause of the migratory grasshopper, *Melanoplus sanguinipes* (Orthoptera: Acrididae). J. Theor. Biol. 112: 827-838.

Hollander, M. and D. A. Wolfe. 1973. Nonparametric statistical methods. Wiley, New York.

Holtzer, T. O., J. R. Bradley, Jr. and R. L. Rabb. 1976. Effects of various temperature regimes on the time required for emergence of diapausing *Heliothis zea.* Ann. Entomol. Soc. Am. 69: 257-260.

Johnson, P. C., D. P. Mason, S. L. Radke and K. T. Tracewski. 1983. Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), egg eclosion: degree-day accumulation. Environ. Entomol. 12: 929-932.

Law, M. A. and W. D. Kelton. 1982. Simulation modeling and analysis. McGraw-Hill, New York.

Logan, J. A., D. J. Wollkind, S. C. Hoyt and L. K. Tanigoshi. 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5: 1133-1140.

Logan, J. A., R. E. Stinner, R. L. Rabb and J. S. Bacheler. 1979. A descriptive model for predicting spring emergence of *Heliothis zea* populations in North Carolina. Environ. Entomol. 8: 141-146.

Lyons, D. B. and T. J. Lysyk. 1988. Development and phenology of eggs of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), in Ontario, pp. 351-65. In Wallner, W.E. and K.A. McManus [eds.], Lymantriidae: a comparison of features of new and old world tussock moths. USDA Gen. Tech. Rep. NE-123.

Maksimovic, M. 1958. Experimental research on the influence of temperature upon development and the population dynamics of the gypsy moth. Posebno Izdanje Bioloskog Instituta NR Srbije, Vol. 3.

Masaki, S. 1956. The effects of temperature on the termination of diapause in the egg of *Lymantria dispar* Linne. Jpn. J. Appl. Zool. 21: 148-157.

Moore, H.W. 1948. Variations in fall embryological development in three grasshopper species. Can. Entomol. 80: 83-88.

Nordin, J. H., Z. Cui and C.-M. Yin. 1984. Cold-induced glycerol accumulation by *Ostrinia nubilalis* larvae is developmentally regulated. J. Insect Physiol. 30(7): 563-566.

Pantyukhov, G. A. 1964. The effect of low temperatures on different populations of the brown-tailed moth *Euproctis chrysorrhoea* (L.) and the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Orgyidae). Entomol. Rev. 43: 47-55.

Powell, M. J. D. 1964. A method for minimizing a sum of squares on nonlinear functions without calculating derivatives. Comp. J. 7: 303-307.

Regniere, J. 1987. Temperature-dependent development of eggs of *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) and simulation of its seasonal history. Can. Entomol. 119: 717-728.

1990. Diapause termination and changes in thermal responsed during postdiapause development in larvae of the spruce budworm, Choristoneura fumiferana. J. Insect Physiol. 36(10): 727-735.

SAS 1985. SAS user's guide. SAS Institute, Cary, N. C..

Sharpe, P. J. H., G. L. Curry, D. W. DeMichele and D. L. Cole. 1977. Distribution model of organism development times. J. Theor. Biol. 66: 21-38.

Tauber, M. J., C. A. Tauber and S. Masaki. 1986. Seasonal adaptations of insects. Oxford University Press, New York.

Tauber, M. J., C. A. Tauber, J. R. Ruberson, A. J. Tauber and L. P. Abrahamson. 1990. Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. Ann. Entomol. Soc. Am. 83(3): 494-503.

Venkatesh, K. and G.M. Chippendale. 1986. Synthesis and release of proteins from cultured larval fat body of the southwestern corn borer, *Diatraea grandiosella.* Insect Biochem. 16: 917-927.

Wagner, T. L., H. Wu, P. J. H. Sharpe and R. N. Coulson. 1984. Modeling distributions of insect development time: a literature review and application of the Weibull function. Ann. Entomol. Soc. Am. 77: 475-483.

Zaslavski, V. A. 1988. Insect development. photoperiodic and temperature control. Springer, Berlin.

Chapter 4

A model of temperature-dependent depletion of stored triglyceride in gypsy moth eggs during prediapause development: implications for egg survival.

Eggs of the gypsy moth, (*Lymantria dispar* (L.), are laid in midsummer. After a period of morphological development, eggs enter diapause in a condition of late embryonic development for the winter. Eggs hatch in May or June following an incubation period in the spring. Larvae migrate from the egg mass to the top of the object on which they were laid and many larvae disperse even if suitable foliage is available (Doane and McManus 1981). The survival of this non-feeding life-stage is partly dependent on its ability to complete the stage, disperse, and begin feeding before their metabolic reserves are fully depleted.

It has been suspected that successive years of defoliation have an adverse effect on gypsy moth females. Carter et al. (1991) found decreased fecundity when defoliation exceeded 40%. Williams et al. (1990) found that egg viability was negatively correlated with percentage defoliation in nine years with outbreak populations. These findings suggest that larval competition for food produces less vigorous females that lay fewer eggs and these eggs have lower survival rates, perhaps due to lower initial metabolic reserves.

It was observed (see Roberts et al. 1993 for a graphical representation) that gypsy moth populations collapse after winters with a large number of mild days. Williams et al. (1990) showed a negative relationship between maximum February temperatures and egg viability. A possible explanation for these observations is an elevated rate of metabolic reserve utilization due to relatively high winter temperatures. Metabolic rate in nondiapausing individuals is positively influenced by temperature, and Gray et al. (1991) reported that eggs in diapause exhibit a significant metabolic response to temperature.

The specific identity of the metabolic energy for gypsy moth embryogenesis has not been identified. However, Chapman (1982) states that lipids are the major metabolic reserve for embryogenesis in insects, their major advantage over carbohydrate reserves being higher potential energy and more metabolic water per unit weight. Yamashita and Hasegawa (1985) state that Nakasone (1979) found that during embryogenesis of the silkworm, *Bombyx mori*, triglycerides accounted for 60% of the total CO_2 expired, while phospholipid and sterol levels remained unchanged. During embryonic diapause and postdiapause development lipids and carbohydrates are the main substances utilized for metabolic energy in Insecta (Yamashita and Hasegawa 1985). Chino (1958) suggested that lipids are the major metabolic energy source during diapause of the silkworm *B. mori*. Lipids were the principal metabolic reserve of diapausing southwestern cornborer, and triglycerides made up over 90% of the fat body lipids (Chippendale 1973).

Therefore, triglycerides likely play a major role in the survival of developing gypsy moth eggs. The objectives of this study were to: 1) quantify the levels of triglyceride present in wild population of gypsy moth eggs at oviposition; and 2) quantify the depletion of triglyceride reserves in individual gypsy moth eggs during prediapause development at a range of constant temperatures.

Materials and Methods

Gypsy moth pupae were collected from a 2 year old infestation in Shenandoah National Park, VA in July 1991 and placed in a 25°C environmental chamber in the quarantine facility of Virginia Polytechnic Institute and State University. Emerging moths were mated, allowed to oviposit, and 4 egg masses were randomly selected. Immediately following oviposition 15 eggs were randomly selected from each egg mass, each egg was weighed, and sacrificed to provide an estimate of initial triglyceride content. An additional 30 eggs were randomly selected from each egg mass and 5 eggs

from each mass were placed in each of 6 environmental chambers at constant temperatures of 5, 10, 15, 20, 25 and 30°C. Respiration rates were measured on individual developing eggs every second day until completion of prediapause was indicated by a decline in respiration rate (Gray et al. 1991). Eggs were sacrificed upon prediapause completion and stored at -70°C until triglyceride content was measured. Human error prevented reliable lipid extraction from 81 eggs. Therefore, estimates of triglyceride content at oviposition and prediapause completion were restricted to 30 and 69 individuals, respectively.

Lipids were extracted from eggs by a double washing with water, chloroform, and methanol (Folch and Sloane-Stanley 1956, Judge et al. 1989). Each egg was ground in a micro centrifuge tube in equal volumes of double-distilled H_2O and a high performance thin layer chromatography (HPTLC) analytical grade chloroform:methanol (2:1) solution. The mixture was vortexed for 2 min, centrifuged at 100 x G for 4 min and the aqueous layer and aqueous-organic interface were removed by pipette to a second tube and rewashed with 400 µl of the chloroform:methanol solution. The aqueous and aqueous-organic mixture was vortexed and centrifuged as above. The organic layers in each centrifuge tube were combined and concentrated under a nitrogen stream, and the volume was adjusted to 10 µl with chloroform.

Silica gel plates (Merck No. 5631) were prewashed with a chloroform:methanol (2:1) solution and activated by placing them at 110°C for 2 h. Eight standards of 0.25, 0.5, 1, 2, 4, 6, 8, and 10 µg/µl mono-, di-and tristearin, cholesterol, and cholesterol palmitate (Sigma Chemical) were prepared. One µl of the lipid extract and 1 µl of each standard were placed on a plate using a CAMAG Nanomat I Automatic Spotter. Lipids were separated in one direction using two development steps. The first development used a benzene:diethyl ether:ethanol:acetic acid (50:40:2:0.5) solution (Freeman and

West 1966). The second development used hexane:ether (7:3) (Judge 1988). The plates were dried with forced air following each development.

Following the second development, plates were dipped in 6-*p*-toludino-2-napthalene-sulfonic acid (1% in methanol), and dried with forced air. The triglyceride spots were quantified using a CAMAG II scanner/densitometer in the fluorescence mode with a Hg lamp, 366 nm wavelength. A standard curve was prepared by regressing the density of the triglyceride standards on their concentrations. Triglyceride content of each egg was estimated by comparing the spot density to the standard curve and correcting for sample dilution.

Data were tested for normality by the Shapiro-Wilk W statistic (SAS Institute, 1990). The correlation between weight and estimated triglyceride content of the 30 eggs that were sacrificed at oviposition was examined with Kendall's distribution-free test for independence (Hollander and Wolf 1973) as a potential means of improving the estimate of initial triglyceride content of the 69 eggs reared through prediapause. The influence of egg mass on estimates of initial triglyceride content among the 30 eggs was also examined by a Kruskal-Wallis test (Hollander and Wolf 1973). The absence of a significant correlation and absence of a significant effect of egg mass (see Results) dictated that estimates of initial triglyceride content of the 69 eggs reared through prediapause be based solely on the mean triglyceride content of the 30 eggs sacrificed at oviposition.

Total triglyceride depletion during prediapause was estimated for each egg by subtracting the content at prediapause completion from the estimated initial content. Confidence intervals of median depletion at each temperature were obtained using Tukey's method (Hollander and Wolf 1973). The distributions of estimated depletion at

each temperature were subjected to pair-wise comparison using a Kolmogorov-Smirnov test (Stephens 1977).

Prediapause developmental rate was estimated for each egg as the inverse of the duration of prediapause (days^{-1}). The rate of triglyceride depletion was estimated for each egg by dividing the estimated total depletion by the duration of prediapause. The correlation between rate of development and rate of triglyceride depletion within each temperature treatment, and for all temperatures together, was examined with Kendall's distribution-free test for independence (Hollander and Wolf 1973).

A temperature-dependent description of developmental rate in prediapause was estimated by fitting

$$R_D(T) = \alpha\left[\left(1 + ke^{-\rho T}\right)^{-1} - e^{-\tau}\right] \qquad [1]$$

to the median developmental rates, where $R_D(T)$ is the developmental rate at T°C above the 10°C base temperature, $\alpha = \rho/\gamma$, $k = (\alpha - \psi)/\psi$, and $\tau = (T_M - T)/\Delta_T$. Parameter ψ is interpreted as the developmental rate at base temperature, ρ as the rate of increase to optimum temperature, γ as a nonlinear effect for enzyme heat denaturization T_M as the lethal, maximum temperature in degrees above the base temperature, and Δ_T as the width of the high level boundary layer (i.e. the temperature span over which the developmental rate function changes its behavior) (Logan et al. 1976).

A temperature-dependent description of triglyceride depletion rate in prediapause was estimated by fitting

$$R_{TG}(T) = \alpha\left[\left(1 + ke^{-\rho T}\right)^{-1} - e^{-\tau}\right] \qquad [2]$$

to the median depletion rates, where $R_{TG}(T)$ is the depletion rate at T°C above the 10°C base temperature, ψ is interpreted as the depletion rate at base temperature, and the remaining variables are as described above.

Estimates of population variability in triglyceride content at oviposition and at prediapause completion were compared by calculating relative volume of triglyceride in each egg. Each estimate of triglyceride content at oviposition was divided by the median estimate at oviposition. Each estimate at prediapause completion was divided by the median estimate at prediapause completion in the same temperature treatment. The relative volumes at oviposition and at prediapause completion were divided into 19 and 33 classes, respectively, of size 0.1 x median. Descriptions of population variability in triglyceride levels at oviposition and prediapause were derived by fitting a modified logistic equation (Regniere, 1984),

$$Y = \left\{1 + \left[e^{-\gamma(X-1)}\right]\left(0.5^{-\alpha} - 1\right)\right\}^{-\alpha^{-1}} \quad , \qquad [3]$$

to the cumulative probabilities of the represented classes. Y is the cumulative probability of X, the upper limit of the class; γ describes the uniformity of the population; and α describes the skewness of the distribution.

Descriptions of population variability in developmental rate were derived in a similar manner. Each developmental rate was divided by the median developmental rate of the same temperature treatment and 22 classes of size 0.025 x median were obtained for the relative rates. All non-linear curve fitting was done using PROC NLIN (SAS, 1990).

Results

Many subsets of the data failed to satisfy the test for normality ($p < 0.05$). Therefore, nonparametric statistical procedures were judged to be more reliable than their parametric counterparts. All combinations of positive or negative skewness with lepto- or playtkurtosis were observed. The manner of departure from normality was inconsistent and precluded any conclusions regarding the distributions.

Mean (± S.E.) live body weight of all eggs at oviposition was 826.7 (± 20.41) μg. The mean (± S.E.) triglyceride content of the 30 eggs sacrificed at oviposition was 117.4 (10.0) μg, 14.2% of live body weight. Median triglyceride content of these 30 individuals was 98.66 μg.

There was no significant correlation (τ = -0.07) between live body weight and triglyceride content at oviposition (KS = -0.55, p = 0.58), nor a significant difference (H = 1.11, p = 0.80) among egg masses in triglyceride content at oviposition. Therefore, the initial triglyceride content of the 69 individuals that were reared to prediapause completion was estimated to be equal to the mean triglyceride content of the 30 individuals from the four egg masses that were sacrificed at oviposition.

Completion of prediapause development at 10, 15, 20, 25, 30, and 35°C reduced mean (± S.E.) triglyceride reserves to 23.3 (3.1), 25.7 (5.5), 32.6 (4.8), 33.1 (4.4), 30.8 (4.1),and 26.3 (6.5) μg, respectively. Median weight (μg) of triglyceride reserves utilized was 92.35 over 53 d at 10°C, 98.8 over 45 d at 15°C, 92.9 over 26 d at 20°C, 86.1 over 16 d at 25°C, 86.2 over 14 d at 30°C, and 93.4 over 14 d at 35°C. Temperature had no significant effect on the amount of triglyceride utilized during prediapause as evidenced by the estimates of median values (Fig. 4.1). Pair-wise comparison of the empirical distribution functions of triglyceride depletion failed to show a significant difference ($p \geq 0.20$) between any pair.

The relationship between median developmental rate in prediapause and temperature was well explained (R^2=.97) by equation [1] except in the lower temperature range. Parameter values are α = 0.095, k = 7.483, ρ = 0.186, T_M = 37.01, and Δ_T = 7.192 (Fig. 4.2). Developmental rates described by this function show an increasing trend over most of the temperature range examined. A near-maximum developmental rate occurs at 30 - 35°C, and rates are predicted to decline sharply above 35°C. The population was quite

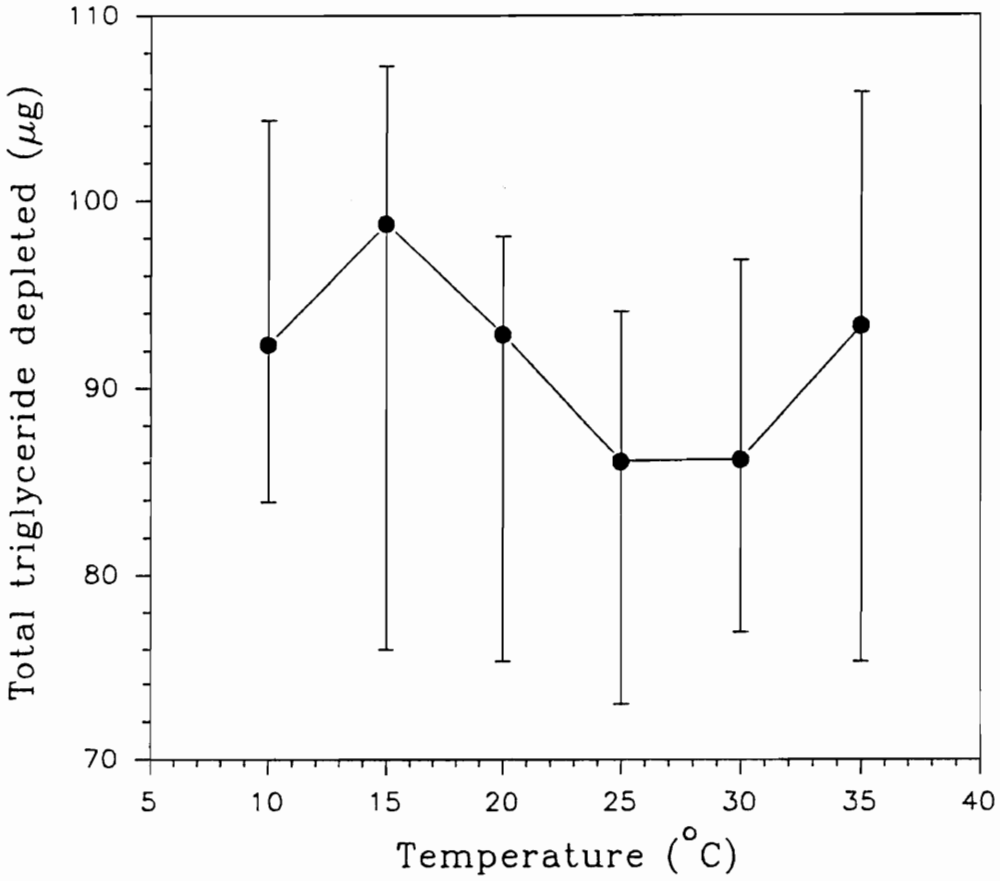

Fig. 4.1. The relationship between observed (●) median (± 95% C.I.) depletion of triglyceride reserves during prediapause and temperature.

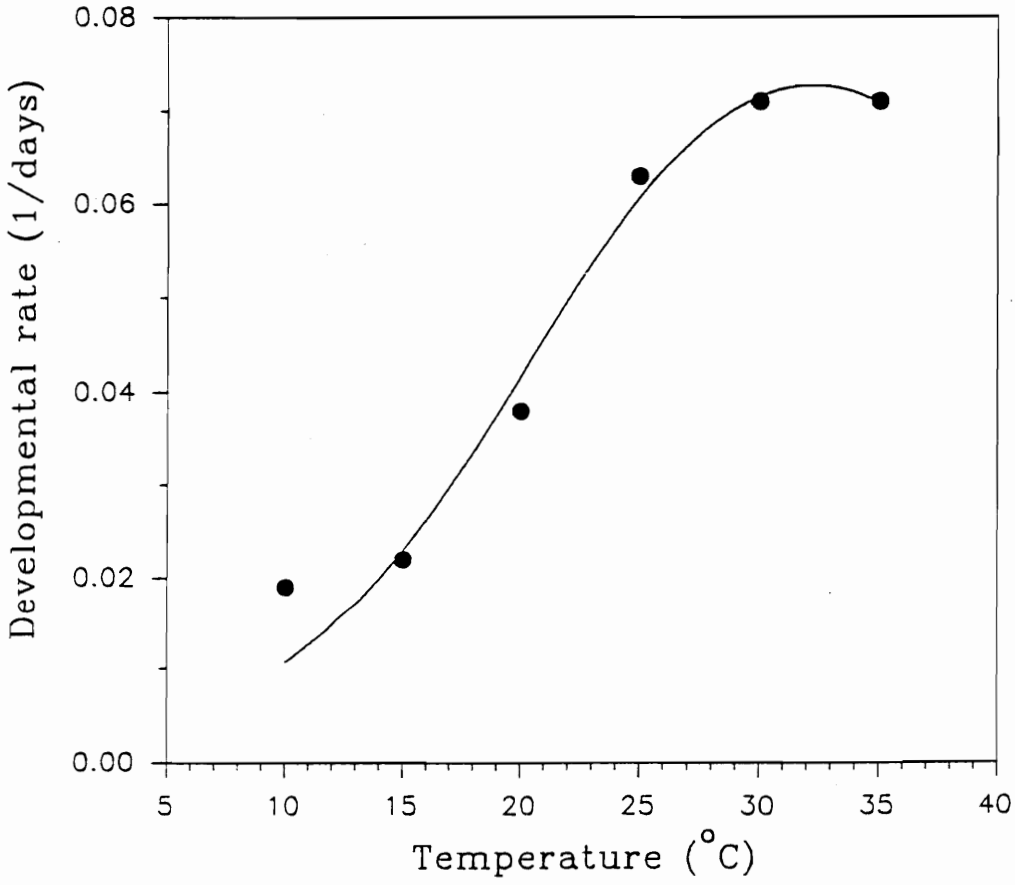

Fig. 4.2. The relationship between observed (●) median developmental rate (R_D) and temperature (T); and the relationship (——) as estimated by: $R_D(T) = \alpha\left[\left(1+ke^{-\rho T}\right)^{-1} - e^{-\tau}\right]$. $\alpha = 0.095$; $k = 7.483$; $\rho = 0.186$; $T_M = 37.01$; $\Delta_T = 7.192$; $R^2 = .97$

uniform in prediapause developmental rate. Equation [3] describes 98% of the observed variability and predicts that ca. 90% of the population has a developmental rate within 10% of the median rate (Fig. 4.3).

The relationship between median rate of triglyceride depletion and temperature was well described (R^2=.98) by equation [2]. Parameter values are $\alpha = 9.550$, $k = 4.965$, $\rho = 0.1234$, $T_M = 54.696$, and $\Delta_T = 13.167$. In contrast to the estimated relationship between development rate and temperature, the rate of triglyceride depletion does not appear to reach a maximum (first derivative is never zero) in the temperature range examined (Fig. 4.4). Thus, increasing temperatures up to approximately 30°C shorten the prediapause duration (increase the developmental rate) and simultaneously increase the rate of triglyceride depletion. Between 30 and 35°C prediapause duration remains relatively constant while the rate of triglyceride depletion shows a modest increase. This simultaneous effect of temperature on prediapause duration and triglyceride depletion rate is further evidenced by the positive correlation ($\tau = 0.65$) between developmental rate and depletion rate ($KS = 8.31$,. $p < 0.00001$) (Fig. 4.5), and the absence of an effect of temperature on total triglyceride depletion (duration x depletion rate) (Fig. 4.1). Within individuals reared at 20°C there was a significant, but weak ($KS = 2.092$, $p < 0.02$), positive correlation ($\tau = 0.37$) between developmental rate and depletion rate. Within individuals reared at the other temperatures there was no significant correlation.

Variability of the population in triglyceride content increased between oviposition and prediapause completion (Fig. 4.6). Equation [3] overestimated the probability of occurrence of low relative triglyceride content in the population even though the coefficients of determination were high for the descriptions of variability in triglyceride content at oviposition ($R^2 = 0.95$) and at diapause completion ($R^2 = 0.99$). However, the

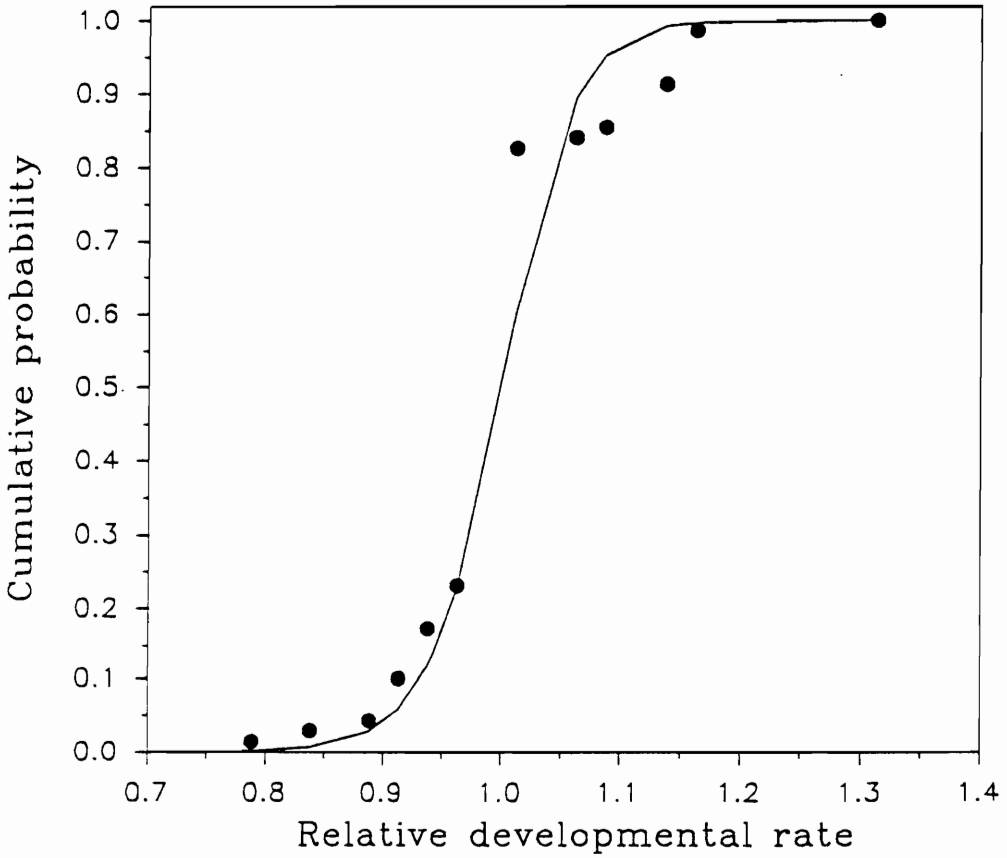

Fig. 4.3 Observed (●) cumulative frequency distribution of relative developmental rate; and the estimated (——) cumulative frequency distribution of relative developmental rate as estimated by:

$$Y = \left\{1 + \left[e^{-\gamma(X-1)}\right]\left(0.5^{-\alpha} - 1\right)\right\}^{-\alpha^{-1}}.\ \gamma = 35.22;\ \alpha = 1.18;\ R^2 = .98$$

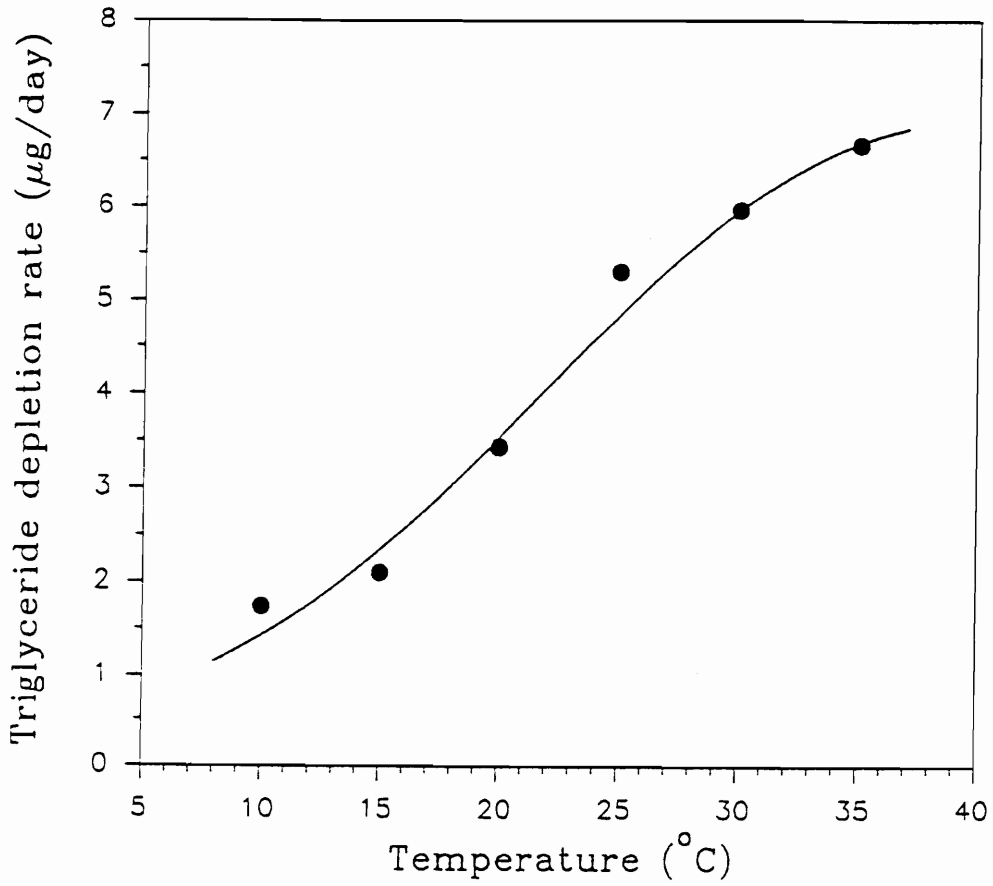

Fig. 4.4. The relationship between observed (●) median triglyceride depletion rate (R_{TG}) and temperature (T); and the relationship (——) as estimated by: $R_{TG}(T) = \alpha\left[\left(1+ke^{-\rho T}\right)^{-1} - e^{-\tau}\right]$. $\alpha = 9.550$; $\rho = 0.1234$; $k = 4.965$; $\Delta T = 13.167$; $T_M = 54.696$; $\tau = {(T_M - T)}/{\Delta_T}$; $R^2 = .983$

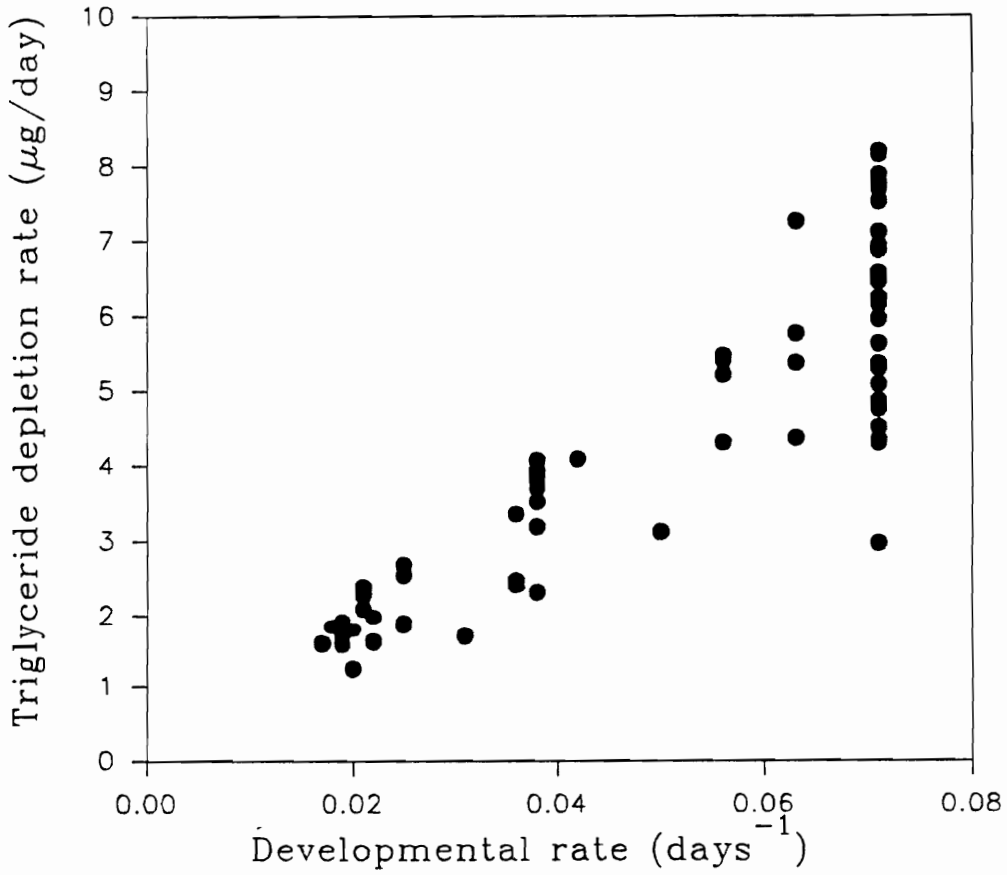

Fig. 4.5. Relationship between observations (●) of triglyceride depletion rate and developmental rate.

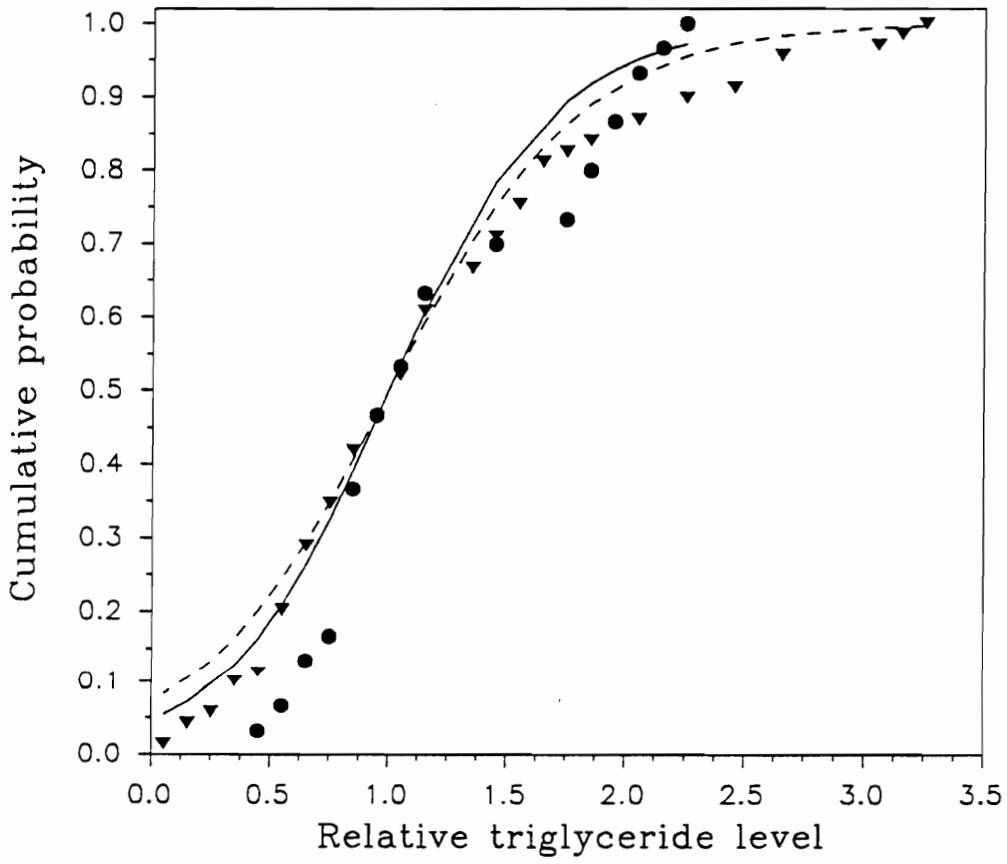

Fig. 4.6. Observed cumulative frequency distribution of relative triglyceride levels at oviposition (●) and prediapause completion (△); and the estimated cumulative frequency of relative triglyceride levels at oviposition (——) and prediapause completion (— —) as estimated by: $Y = \left\{1 + \left[e^{\gamma(X-1)}\right]\left(0.5^{-\alpha} - 1\right)\right\}^{-\alpha^{-1}}$. Parameters at oviposition: $\gamma = 2.80$; $\alpha = 0.89$; $R^2 = .98$. Parameters at prediapause completion: $\gamma = 2.44$; $\alpha = 0.94$; $R^2 = .99$

conclusion of an increase in variability is supported by both a visual examination of the data and by a comparison of γ for oviposition (2.8) vs prediapause completion (2.4).

Discussion

Temperature has a profound effect on duration of development and rate of triglyceride depletion during gypsy moth prediapause. However, there is no significant correlation between developmental rate and depletion rate within individual temperatures (except 20°C). The absence of this association causes there to be no effect of temperature on total depletion (Fig. 4.1), as opposed to the effect that would result if individuals with median developmental rates also experienced the median rate of depletion (Fig. 4.7). In addition, the increase in variability between relative triglyceride reserves at oviposition and prediapause completion (Fig. 4.6) indicates that eggs that initiate prediapause with smaller triglyceride reserves do not completely compensate by utilizing less during prediapause. Thus a relatively uniform quantity of triglycerides are utilized during prediapause development, regardless of temperature or initial levels.

The relatively uniform total depletion among individual eggs at different temperatures suggests that the completion of prediapause, and the initiation of diapause, occurs when a certain volume of triglyceride has been utilized. Bell (1989) stated that embryos appear as fully differentiated larvae a few days before the onset of diapause. Triglycerides are a likely source of metabolic energy for embryogenesis (Chapman 1982). Yamashita and Hasegawa (1985) stated that Nakasone (1979) found that during *B. mori* embryogenesis triglycerides accounted for 60% of the total CO_2 expired. Thus, individuals could be expected to utilize a given volume of triglycerides, regardless of temperature, in completing a given amount of embryological development.

Triglycerides may be further reduced in the preparation for diapause beyond their utilization in embryogenesis. Pantyukhov (1964) reported that gypsy moth eggs had

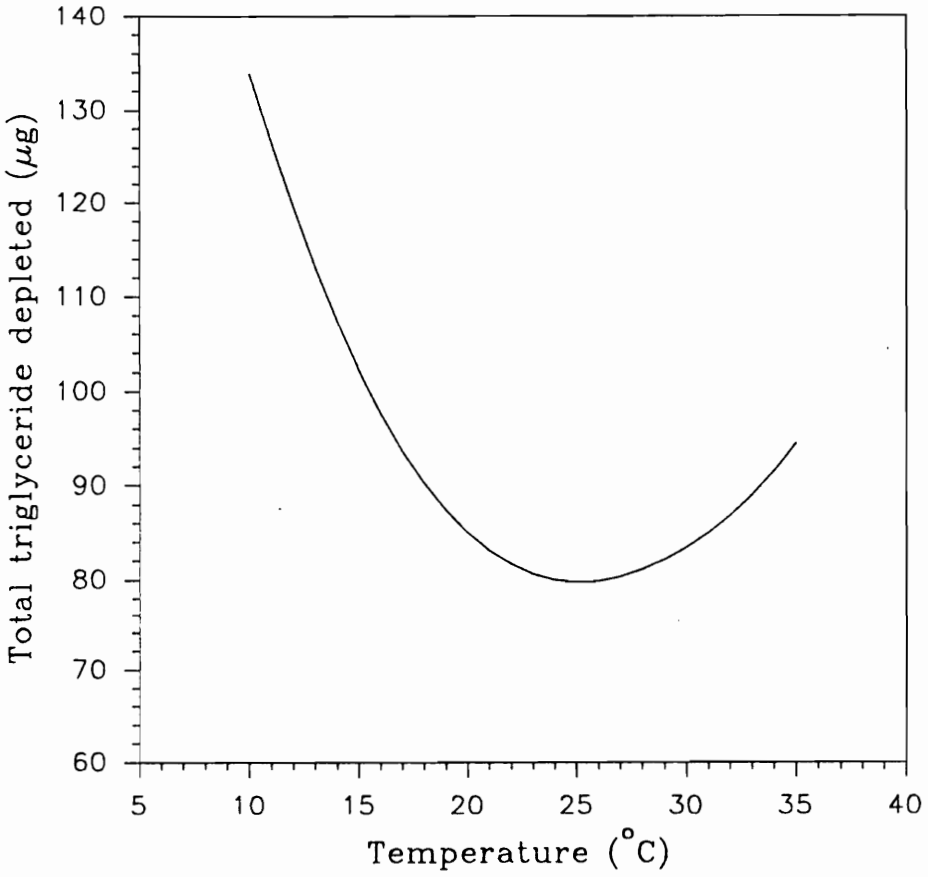

Fig. 4.7. The relationship between total triglyceride depleted and temperature as derived from equation [1] (Fig. 4.2) and equation [2] (Fig. 4.3).

supercooling points as low as -20°C in early diapause before exposure to cold temperatures. Supercooling capabilities are most often associated with elevated levels of polyhydric alcohols such as glycerol (Lee 1991). Glycogen is the most common substrate for polyol synthesis (Chino 1958, Mansingh 1974, Lee 1991), but Mansingh and Smallman (1972) suggest that some glycerol synthesis uses a non-carbohydrate substrate, probably lipids. The production of cryprotectants occurs independent of temperature in species where diapause and cold hardiness are linked (Denlinger 1991). Thus, it could be expected that a uniform quantity of cryoprotectants would be produced at any temperature experienced during prediapause.

The very large proportion (73% to 84%) of triglyceride reserves utilized during prediapause may have significant consequences for egg hatch and larval survival. Chino (1958) and Nakasone (1979) found evidence that lipids, probably triglycerides, are the major metabolic energy source during diapause of *B. mori*. Prediapause results in a uniform depletion of triglycerides, probably because the phase is completed when a given level of embryogenesis has been completed. However, the volume of triglycerides utilized during diapause may be affected by temperature. While it is known that metabolic activity is minimal during diapause, metabolic reserves are still necessary to survival (Harvey 1962). And Gray et al. (1991) provided evidence that gypsy moth eggs in diapause remain responsive to temperature. While they showed that increasing temperatures cause increasing respiration rates, it has been shown by others (Masaki 1956, see Giese and Cittadino 1977 for a general review) that diapause development is hindered by temperatures above 15°C. Thus, high temperatures may prolong the diapause phase while simultaneously increasing the demand on metabolic reserves. Eggs that enter diapause with low metabolic reserves will be less likely to survive. It has been observed that the advancing infestation in Virginia receded in 1990 following a warm

winter (see Roberts et al. 1993 for representation of pheromone trap catches) and Williams et al. (1990) reported a negative relationship between maximum February temperatures and egg viability over a 9 year period. Excessive depletion of triglyceride reserves may lead to post-hatch death. Many gypsy moth neonates disperse before feeding (Doane and McManus 1981).

The volume of metabolic reserves deposited in eggs may be influenced by the vigor of females. Williams et al. (1990) found an inverse relationship between defoliation and egg viability, suggesting that larval competition for food adversely affected the physiology of female moths and their progeny. Carter et al. (1991) found fecundity to be adversely affected when defoliation exceeded 40%. The amount of triglyceride deposited in newly oviposited eggs may have a serious impact on egg hatch and neonate survival. For example, if 40% defoliation reduces initial triglyceride reserves by only 10% (from 117.4 μg), and prediapause depletion equals 90 μg, the triglyceride level at prediapause completion level will be reduced to 57% of the level under undefoliated conditions.

Future work should examine the potential effect of temperature on utilization of metabolic reserves during diapause and postdiapause development, and the impact on egg hatch and survival of neonate larvae.

Literature Cited

Bell, R. A. 1989. Respiratory activity during embryonic development in a diapausing and a selected non-diapausing strain of the gypsy moth, *Lymantria dispar* L. Comp. Biochem. & Physiol. 93A: 761-771.

Carter M. R., F. W. Ravlin, and M. L. McManus 1991 Changes in gypsy moth (Lepidoptera: Lymantriidae) fecundity and male wing length resulting from defoliation. Environ. Entomol. 20 (4): 1042-1047.

Chapman, R. F. 1982. The insects. Harvard University Press, Cambridge, Massachesetts. 919 pp.

Chino H. 1958. Carbohydrate metabolism in the diapause egg of the silkworm, Bombyx mori - II: conversion of glycogen into sorbitol and glycerol during diapause. J. Insect. Physiol. 2: 1-12.

Chippendale, G. M. 1973. Diapause of the southwestern corn borer, Diatraea grandiosella: utilization of fat body and haemolymph reserves. Entomol. Exp. Appl. 16:395-406.

Denlinger, D. L. 1991. Relationship between cold hardiness and diapause. *In* R. E. Lee and D. L. Denlinger [eds.], Insects at low temperatures. Chapman and Hall, New York.

Doane, C. C., and M. L. McManus. 1981. The gypsy moth: research toward integrated pest management. USDA For. Serv. Tech. Bull. 1584.

Folch J., and G. M. Sloane-Stanley 1956. A simple method for the isolation and purification of total lipids form animal tissues. J. Biol. Chem. 226:497-509.

Freeman C. P., and D. West 1966. Complete separation of lipid classes on a single thin-layer plate. J. Lipid Res. 7: 324-327.

Giese, R. L. and M. L. Cittadino. 1977. Relationship of the gypsy moth to the physical environment. II. Diapause. Staff Paper Series No. 6. Dept. of Forest., U. of Wis. 13 pp.

Gray D. R., J. A. Logan, F. W. Ravlin and J. A. Carlson 1991. Toward a model of gypsy moth egg phenology: Using respiration rates of individual eggs to determine temperature-time requirements of pre-diapause development. Environ. Entomol. 20: 1645-1652.

Harvey W. R. 1962. Metabolic aspects of insect diapause. Ann. Rev. Entomol. 7: 57-80.

Hollander, M. and D. A. Wolfe. 1973. Nonparametric statistical methods. Wiley, New York.

Lee, R. L. 1991 Principles of insect low temperature tolerance. *In* R. E. Lee and D. L. Denlinger [eds.], Insects at low temperatures. Chapman and Hall, New York.

Judge, D. N. 1988. Flight activity and hemolymph diacylglyceride concentrations in *Heliothis zea* (Bodie) (Lepidoptera: Noctuidae). M.Sc. Thesis. Virginia Polytechnic Insitute and State University, Blacksburg, VA. 110 pp.

Judge, D. N. , D. E. Mullins and J. L. Eaton. 1989. Microquantity analysis of insect hemolymph lipids by high performance thin layer chromatography. J. Planar Chrom.2:442-446.

Logan J. A., D. J. Wollkind, S. C. Hoyt, and L. K Tanigoshi. 1976. An analytic model for description of temperature dependent rate phenomena in arthropods. Environ. Entomol. 5: 1133-1140.

Mansingh, A. 1974. Studies on insect dormancy. II. Relationship of cold-hardiness to diapause and quiescence in the eastern tent caterpillar, *Malacosoma americanum* (Fab.), (Lasiocampidae: Lepidoptera). Can. J. Zool. 52: 629-637.

Mansingh, A. and B. N. Smallman. 1972. Variation in polyhydric alcohol in relation to diapause and cold-hardiness in the larvae of *Isia isabella*. J. Insect Physiol. 18:1565-1571.

Masaki, S. 1956. The effects of temperature on the termination of diapause in the egg of *Lymantria dispar* Linne. Jpn. J. Appl. Zool. 21: 148-157.

Nakasone S. 1979. Changes in lipid components during the embryonic development of the silkworm, *Bombyx mori*. J. Sericult. Sci. Japan 48: 526-532. (In Japanese with English summary).

Pantyukhov, G. A. 1964. The effect of low temperatures on different populations of the brown-tailed moth *Euproctis chrysorrhoea* (L.) and the gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Orgyidae). Entomol. Rev. 43: 47-55.

Regniere J. 1984. A method of describing and using variability in development rates for the simulation of insect phenology. Canad. Entomol. 116: 1367-1376.

Roberts, E. A., F. W. Ravlin and S. J. Fleischer. 1993. Spatial data representation for integrated pest management programs. Amer. Entomol. 39 (2): 92-107.

SAS Institute. 1990. SAS/STAT user's guide. Ver. 6. Vol. 2. SAS Institute, Cary, NC.

Stephens, M. A. 1977. EDF statistics for goodness of fit and some comparisons. Technometrics 19:205-210.

Williams R. D., R. W. Fuester, W. W. Metterhouse, R. J. Balaam, R. H. Bullock, R. J. Chianese, and R. C. Reardon 1990. Density, size, and mortality of egg masses in New Jersey populations of the gypsy moth (Lepidoptera: Lymantriidae). Environ. Entomol. 19: 943-948.

Yamashita O and. Hasegawa K. 1985. Embryonic diapause. *In* Comprehensive Insect Physiology, Biochemistry, and Pharmacology (Edited by Kerkut G. A. and Gilbert L. I.). Vol. 1, pp 408-434. Pergamon Press, Oxford.

Chapter 5

Further advances toward a model of gypsy moth egg phenology: respiration rates and thermal responses during diapause and age-dependent developmental rates in postdiapause

Existing simulation models of gypsy moth 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 non-linearly (Lyons and Lysyk 1989, Waggoner 1984) dependent on temperature. These models were fit 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 , pers. comm., 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 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 McManus (unpublished) as evidence for a discrete phase transition between diapause and postdiapause occurring after ca. 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* (Bt) and nucleopolyhedrosis virus (NPV) over diverse landscapes (Schaub et al. 1994); dispersion of egg masses from "substerilized" gypsy moth adults (Mastro et al. 1989); and conducting detection surveys.

In this chapter I report on thermal responsiveness (sensu Gray et al. 1991) and on changes in the pattern of respiration rate of gypsy moth eggs as an indication of the

transition between diapause and postdiapause. I also analyze and model changes in the developmental rate response to temperature that occur during postdiapause.

Materials and Methods

Gypsy moth eggs. Gypsy moth 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) after they had experienced 25°C, and a 16:8 (L:D) 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 in order to advance the diapause phase as needed for Experiments 1 and 2 (see below). All egg masses continued development in my laboratory at 5°C and 16:8 (L:D) photoperiod until noted in the experiments described.

Respiration rate. Estimation of thermal responsiveness requires the measurement of the respiration rates of individual gypsy moth eggs. Respiration rates were determined by placing eggs in individual 1-ml glass autosampler vials (Fisher Scientific, Pittsburgh, PA, 03-340-5A,), 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 *u*l gas sample was withdrawn from each vial using a gas-tight syringe (Supelco, Belefonte, PA, 2-0739) and injected into an open-system infrared gas analyzer (IRGA) (LI-6251, Li-Cor Inc., Lincoln, NB). A 250 *u*l 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 (mvolts) 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. 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 five 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.

Experiment 1: Thermal Responsiveness During Diapause and Postdiapause Phases. Two egg masses in each of 10 classes of relative age experienced 5°C and a 16:8 (L:D) 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. Approximately every third day 15 eggs from each egg mass were removed from the 5°C treatment, placed in vials and 3 eggs from each mass were then placed in each of 5, 10, 15, 20 and 25°C treatments. Eggs were allowed to acclimatize for approximately 18 hours before vials were sealed. Respiration rates were measured and the eggs were discarded. Respiration rates were measured on 9 samples from each egg mass over a 28 day period, resulting in egg mass ages (days in 5°C) ranging from 2 to 155 days.

Gray et al. (1991) defined thermal responsiveness as the change in respiration rate per degree change in temperature ($\Delta\mu$l CO_2/24 h/°C). For the reasons stated in the addendum of Chapter 3, a more accurate definition of thermal responsiveness is the relative change in respiration rate per degree change in temperature ($\Delta R/\%_C$). Relative respiration rates were calculated by dividing each respiration rate by the mean respiration rate at 25° of the same age x egg mass combination. Thermal responsiveness was estimated for each age by linear regression (Zar 1984) of relative respiration rate on temperature.

The relationship between physiological age (days at 5°C) and respiration rate at 25°C was examined by linear regression (PROC REG, SAS 1990). Ranges of physiological 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 Results below) 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 can not 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 age.

Initial examination of the data (see Results below) 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

$$R_T(t) = R_T(0)e^{a_T t} \quad , \qquad [1]$$

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

Physiological age in postdiapause after t days is then

$$A_T(t) = \int_0^t R_T(t)dt = \frac{R_T(0)}{a_T}(e^{a_T t} - 1) \quad . \qquad [2]$$

Developmental rate can be expressed as a time-independent function of physiological age by solving [2] for t ,

$$t = \frac{1}{a}\ln(\frac{a_T A}{R_T(0)} + 1) \quad , \qquad [3]$$

and substituting the result into [1],

$$R_T(A) = R_T(0) + a_T A \quad . \qquad [4]$$

Egg masses used in the following experiments were obtained from Hamden after they had experienced 5°C and a 16:8 (L:D) photoperiod for 100d. Based upon results of Experiment 1 (see Results) this was determined to be a sufficient duration of cold temperature for diapause completion in most egg masses.

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 placed in a 15°C treatment to continue postdiapause development. On days 0, 2, 4 and 6 approximately 25 eggs from each egg mass were placed at each of five temperatures (T = 5, 10, 20, 25 and 30°C) for two to ten days and then returned to 15°C. An additional sample of eggs was transferred from 15°C to 30°C for two 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 x 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 x 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 most egg masses were represented by at least 20 eggs in each temperature x time combination.

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

$$R_T(t) = \frac{1 - \frac{t_{15}}{t_{med15}}}{t_T} \quad \text{(Regniere 1987),} \qquad [5]$$

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 $1 - \frac{t_{15}}{t_{med15}}$, 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 (L:D) photoperiod.

Median values of $R_T(t)$ were estimated from the resulting 200 developmental rates within each temperature x time combination. A saturated model was used in an analysis of covariance (PROC GLM, SAS 1990) to estimate the effect of T, t and the interaction $T*t$ 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, $R_T(t)/\overline{R}_T$. Analysis of covariance examined the effect of T, t and $T*t$ on $R_T(t)/\overline{R}_T$. 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 non-linear fit of $R'(0) + a't$ and $R'(0)e^{a't}$ to $R_T(t)/\overline{R}_T$ where $R'(0)$ and a' are the temperature-independent descriptions of developmental rate at the onset of postdiapause and the amount of change in 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

$$R_T(0) = \tau + \delta T \qquad [6]$$

to these estimates. 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 [6] 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 each divided into six samples and 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. One hundred was chosen as the normalizing factor because most egg masses were represented by at least 100 eggs in each temperature treatment. Median times to hatch were estimated from the resulting 1000 developmental times within each temperature treatment.

Estimates of a_T were obtained by solving [2] iteratively 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 developmental rate at postdiapause initiation obtained from Experiment 2.

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

$$a_T(T) = \omega + \kappa T + \psi T^2 + \vartheta T^3 \quad . \qquad [7]$$

A value of a_5 was not available because gypsy moth eggs do not hatch readily at such low temperatures. However, an increase in 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 [7].

Temperature-dependent descriptions of developmental rate variability of the population were derived by fitting a logistic function (Regniere 1984),

$$F(x^{-1}) = (1 + e^{-\gamma(x^{-1}-\beta)})^{-\alpha^{-1}} \quad , \qquad [8]$$

to the inverse of the 1000 times in each temperature treatment. $F(x^{-1})$ is the cumulative probability of hatch within x days, γ describes the uniformity of the population, β is the time at which 50% of the population will have hatched, and α describes the skewness of the distribution. A temperature-independent description of developmental rate variability was derived by normalizing the inverse times (dividing each inverse by the median inverse of the temperature treatment) and fitting [8] to the normalized inverse times. Curves were fit by PROC NLIN (SAS 1990). Since time to hatch in this experiment is a result of the combined effects of 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. Physiological age (time at 5°C) appeared to have little effect on respiration rate at 25°C for the first 100 days following the onset of diapause (Fig. 5.1). Regression analysis estimated that respiration rate increased 0.0054 (± 0.0004) *u*l CO_2/24 h per day during this period. After day 100 respiration rate increased 0.0486 (± 0.0047) *u*l CO_2/24 h per day.

There was no trend to the estimated thermal responsiveness for the 155 days following the onset of diapause (Fig. 5.2). The mean (± S.E.) estimate of thermal responsiveness was 0.0434 (0.0009) $\Delta R/{}^{\circ}C$. Thus, respiration rates at all

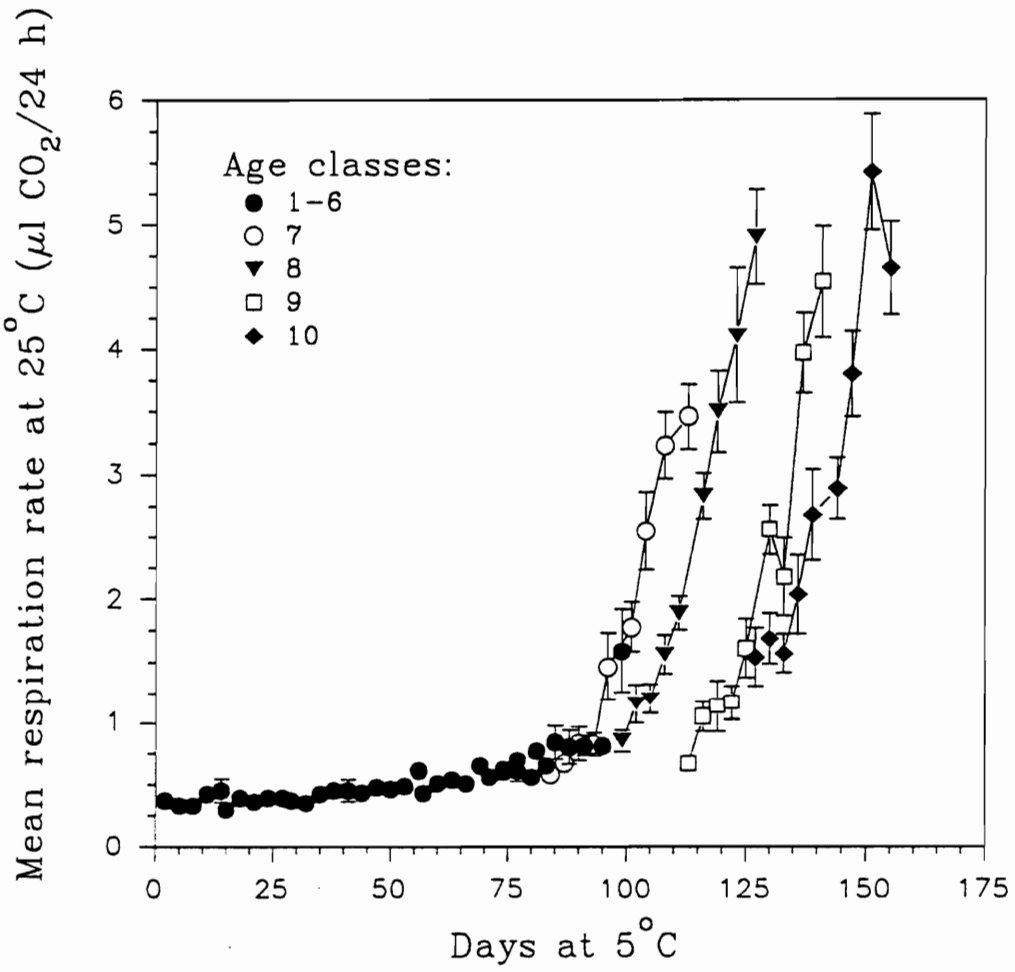

Fig. 5.1. The relationship between mean respiration rate at 25°C (± S.E.) and physiological age (days at 5°C) for 155 d following onset of diapause.

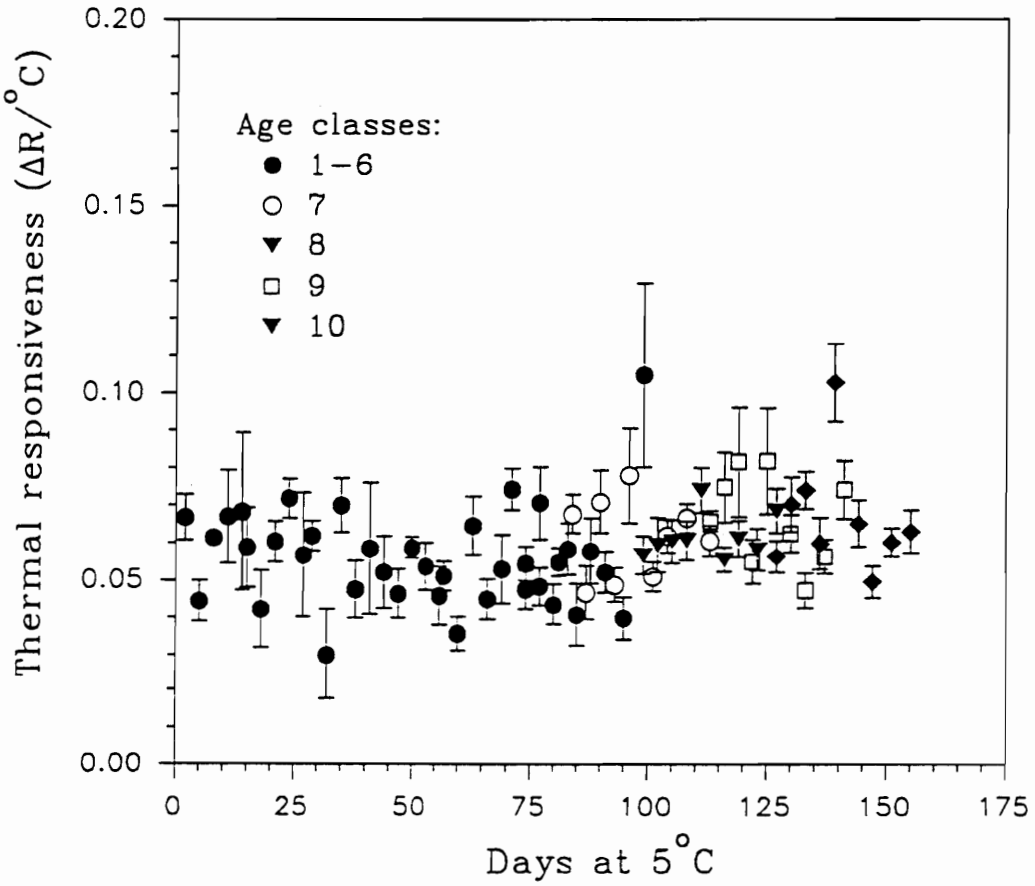

Fig. 5.2. The pattern of thermal responsiveness (± S.E.) for 155 d at 5°C following onset of diapause.

temperatures were affected similarly by physiological age and eggs were equally responsive to temperature throughout the experiment.

Experiment 2: Relationship between R_T and t. A total of 3,985 eggs hatched in Experiment 2. Mean egg hatch per temperature x time combination was 249 (S.E. = 15.6). A minimum of 154 observations occurred in a temperature x time combination from which to calculate the 200 normalized observations. Egg hatch 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 $T*t$ ($F = 19.06$, df = 1,12 $p < 0.001$; Fig. 5.3a). After removing the effect of T by calculating relative developmental rates $R_T(t)/\overline{R}_T$, the analysis of covariance indicated that there was no effect of T or $T*t$, and only t had a significant effect on $R_T(t)/\overline{R}_T$ ($F = 15.44$, df = 1,12, $p < 0.002$). These results indicate that the relative change in developmental rate with increasing physiological age (time at 15°C) is the same regardless of the temperature to which eggs are subsequently exposed (Fig. 5.3b). The relationship between $R_T(t)/\overline{R}_T$ 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 [4].

A total of 1,232 eggs hatched that were transferred from 15°C to the five temperature treatments on day 0. Mean egg hatch per temperature treatment was 246 (S.E. = 7.62). A minimum of 224 observations occurred in a temperature x time combination from which to calculate the 200 normalized observations. 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 [6]

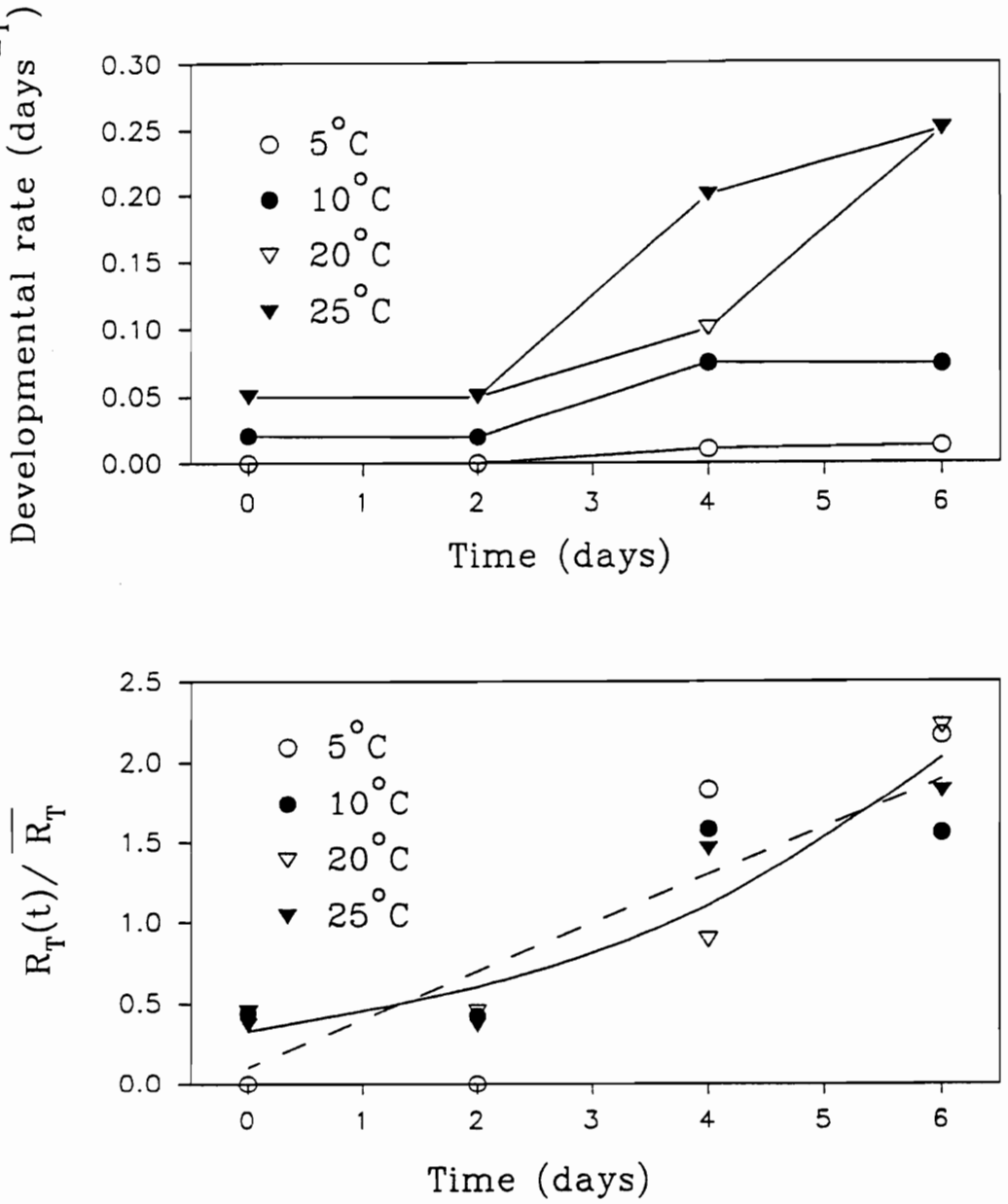

Fig. 5.3. The relationship between postdiapause developmental rate and time at four temperatures (left). The relationship between observed $R_T(t)/\overline{R_T}$ and time for the same four temperatures and the relationship as estimated by a linear (——) and an exponential (— —) function (right). Linear function: $R_T(t)/\overline{R_T} = R'(0)+a't$. $R'(0) = 0.102$; $a' = 0.299$; $R^2 = 0.779$. Exponential function: $R_T(t)/\overline{R_T} = R'(0)e^{a't}$. $R'(0) = 0.303$; $a' = 0.330$; $R^2 = 0.800$

fit the median $R_T(0)$ values well ($R^2 = 0.97$) (Fig. 5.4). Parameter values are given in Table 1. The initial developmental rate $R_{15}(0) = 0.0319$ days^{-1} was estimated with [6].

Experiment 3: Estimation of a_T. A total of 5,762 eggs hatched from the 10 egg masses. Mean egg hatch per temperature treatment was 1,152 (S.E. = 269). 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 [7] fit the estimates of a_T very well ($R^2 = 0.99$). 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 to 30°C temperature range (Fig. 5.5).

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 [8], with R^2 ranging from 0.98 (20°C) to 0.99 (5°C) (Table 1). The single, temperature-independent, estimate of population variability also described the observed variability very well ($R^2 = 0.99$) (Fig. 5.6). 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. 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) each phase is distinct and governed by a unique

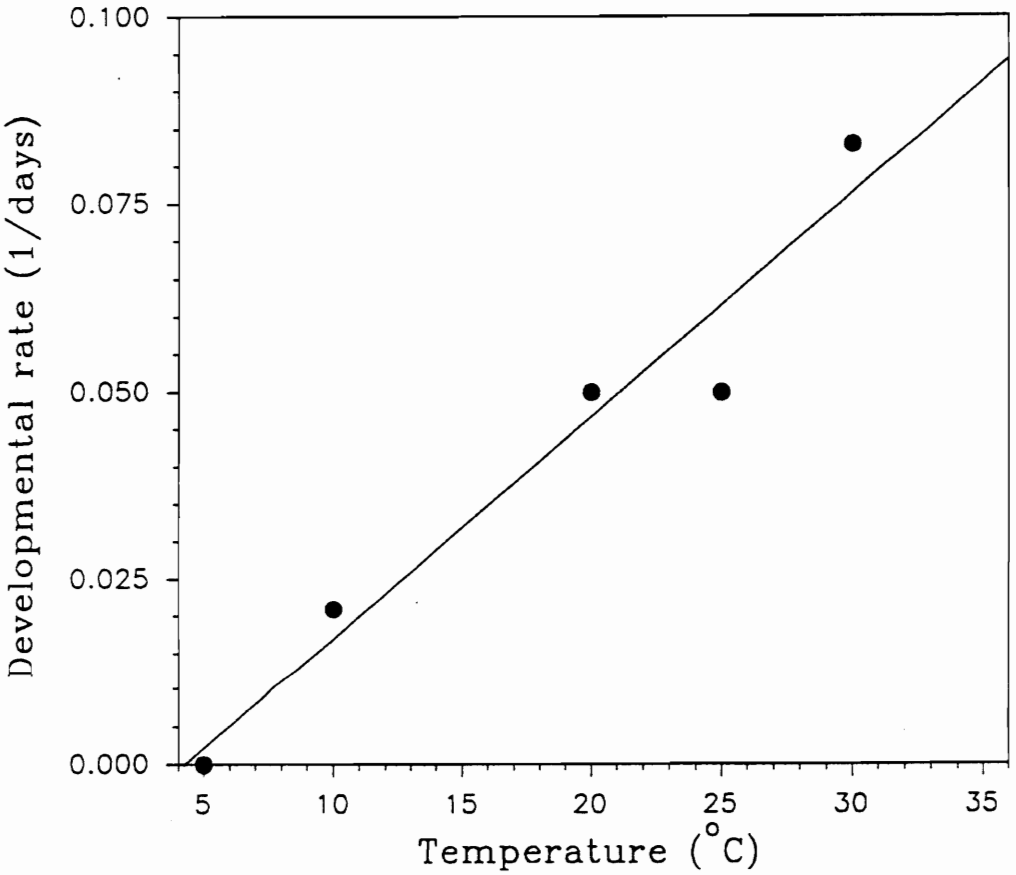

Fig. 5.4. The relationship between observed (●) initial postdiapause developmental rate, $R_T(0)$, and temperature, T; and the relationship as estimated by: $R_T(0) = \tau + \delta T$. τ = -0.0127; δ = 0.00297; R^2 = 0.97

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	β	α	R^2
Initial rate[a]	-0.0127	0.00297								0.97
rate change[b]			-0.08323	0.01298	0.00099	-0.00004				0.99
Pop. variability[c]										
10°C							283.927	0.054	1.253	0.99
15°C							118.296	0.088	0.082	0.99
20°C							92.616	0.120	1.030	0.98
25°C							63.815	0.085	0.202	0.99
30°C							55.439	0.089	0.406	0.99
temp. indep.							8.892	0.909	0.551	0.99

[a] $R_T(0) = \tau + \delta T$

[b] $a_T(T) = \omega + \kappa T + \psi T^2 + \vartheta T^3$

[c] $F(x^{-1}) = (1 + \varepsilon^{-\gamma(x^{-1}-\beta)})^{-\alpha^{-1}}$

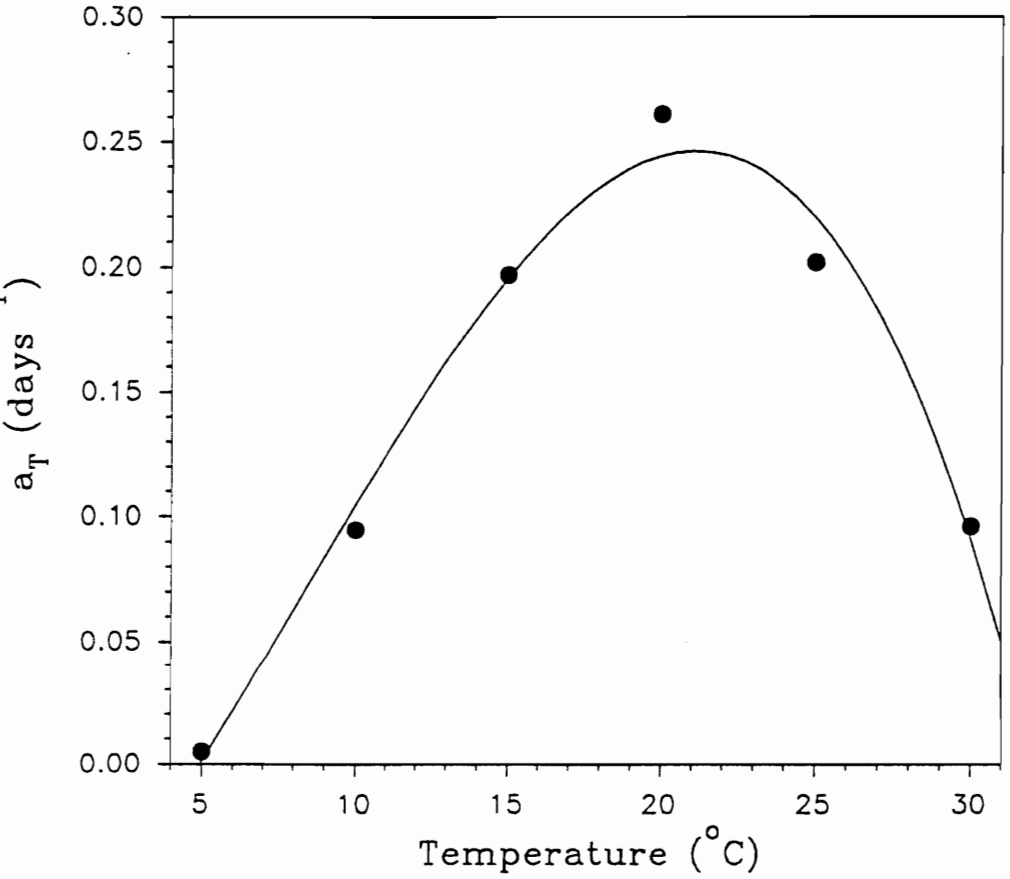

Fig. 5.5. The relationship between the observed (●) postdiapause developmental rate change parameter, a_T, and temperature , T; and the relationship as estimated by: $a_T(T) = \omega + \kappa T + \psi T^2 + \vartheta T^3$.
ω = -0.08323; κ = 0.01298; ψ = 0.00099; ϑ = -0.00004; R^2 = 0.99

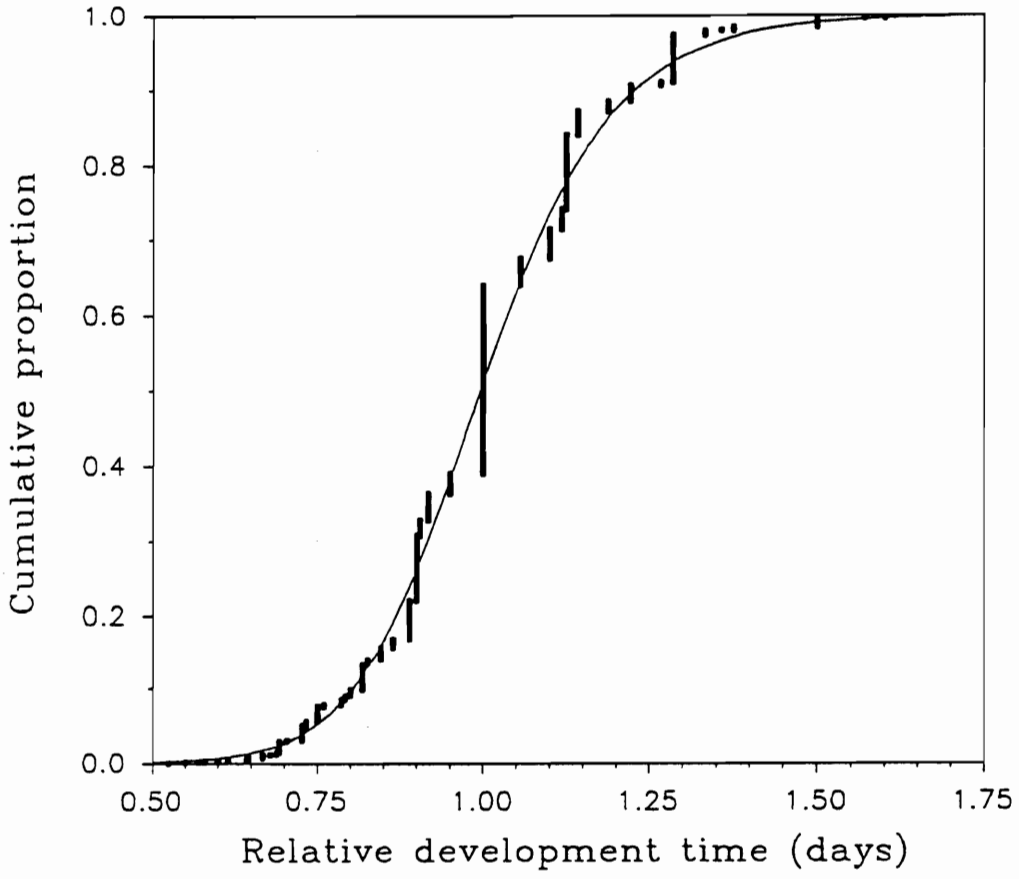

Fig. 5.6. Observed (●) cumulative probability of temperature-independent variability in the composite postdiapause developmental rates; and the variability as estimated by: $F(x^{-1}) = (1+e^{-\gamma(x^{-1}-\beta)})^{-\alpha^{-1}}$. $\gamma = 8.892$; $\beta = 0.909$; $\alpha = 0.551$; $R^2 = 0.99$

temperature-dependent developmental rate response, and there is a clear transition between the phases. In the model of Tauber et al. (1990), further developed by Sawyer et al. (1993), there is no clear transition between diapause and postdiapause and the temperature-dependent developmental response gradually increases with 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. 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 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). This has been amended (Chapter 3) to equal the relative magnitude of the respiration response to a change in temperature. Using this variable it is seen that eggs throughout the diapause and postdiapause phases do not vary in their sensitivity to temperature (Fig. 5.2). However, respiration rates at 25°C strongly suggest that a physiological transition occurs approximately 100 days, at 5°C, after the onset of diapause.

These data clearly display a pattern that can be divided into two phases. During the first phase respiration rate at 25°C remains low and differs only very little with age (0 - 100 days) (Fig. 5.1). An additional day at 5°C during this phase causes respiration rate at 25°C to increase only 0.0054 *u*l CO_2/24 h. During the second phase thermal responsiveness increases dramatically. An additional day at 5°C causes respiration rate at 25°C to increase 0.0486 *u*l CO_2/24 h, approximately 9 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 favorable for development. They equate the diapause state with low thermal responses, and the postdiapause state with higher thermal responses. On this basis I believe that diapause is evident from the low thermal responses observed for the 100 days immediately following diapause initiation (Fig. 5.1).

The dramatic increase in thermal response that was first exhibited 100 days after diapause initiation marks the onset of the postdiapause phase. Eggs from masses that exhibited thermal responses greater than 3.0 *u*l CO_2/24 h, and that were subsequently

held at 25°C, hatched within 3 days. Thus, this phase can not be divided further on the basis of thermal response. Instead, thermal response increases during postdiapause until eggs hatch.

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 age in postdiapause is more accurately modeled with a temperature- and age-dependent function.

Regniere (1990) described an 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 age increased and temperatures of 12 - 24°C had an increasing effect as age increased. Developmental rates were negatively temperature-dependent at young ages and became positively temperature-dependent at more advanced ages. Postdiapause budworm will advance through early physiological ages (ca. 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 an age- and temperature-dependent process where developmental rates are positively age-dependent at all temperatures, and positively temperature-dependent at all ages. However, response is initially slow and relatively insensitive to temperature (Fig. 5.7). 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

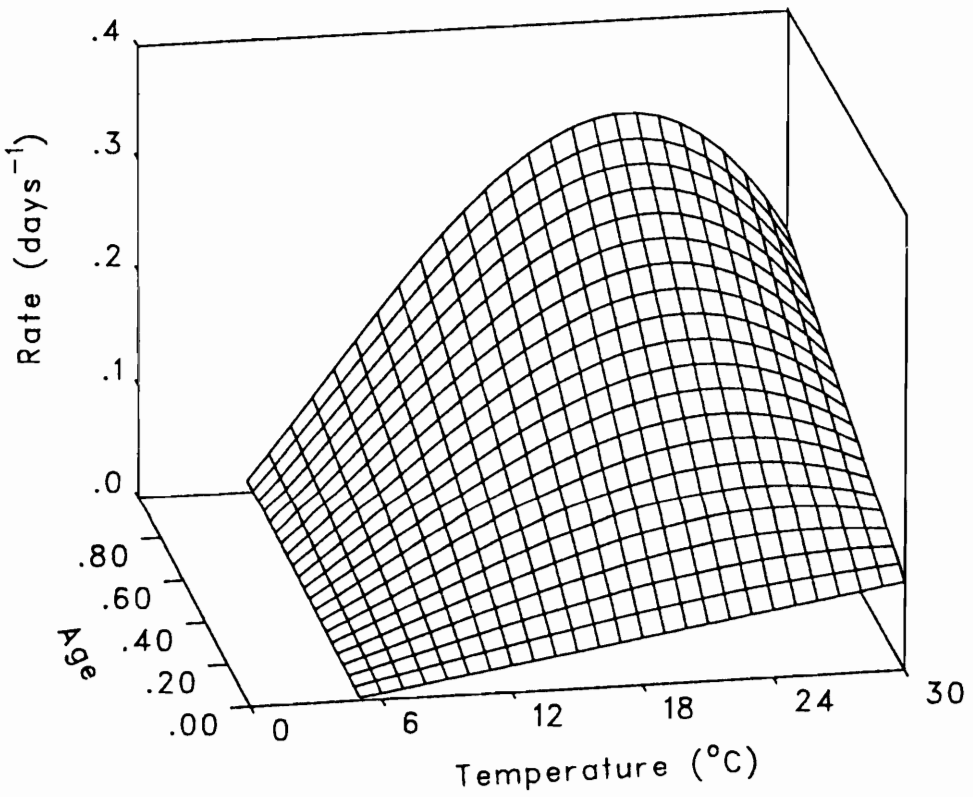

Fig. 5.7. The age-dependent relationship between postdiapause developmental rate and temperature.

later in the spring has a greater effect since eggs are more physiologically advanced. For example, developmental rate at 22°C is 0.05 $days^{-1}$ at postdiapause initiation, and $0.25d^{-1}$ at physiological age 0.75.

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, 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 0.5 and 1.6 times the median. I 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), I 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 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 age-dependent developmental rates of the postdiapause phase.

Literature Cited

Gray, D. R. , J. Regniere, F. W. Ravlin, and J. A. Logan. 1993. A comparison of three egg hatch models in eastern North America. USDA For. Serv. Gen. Tech. Rep. NE-179. 127 pp.

Gray, D. R., J. A. Logan, F. W. Ravlin and J. A. Carlson. 1991. Toward a model of gypsy moth egg phenology: Using respiration rates of individual eggs to determine temperature-time requirements of pre-diapause development. Environ. Entomol. 20(6):1645-1652.

Johnson, P. C., D. P. Mason, S. L. Radke and K. T. Tracewski. 1983. Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), egg eclosion: degree-day accumulation. Environ. Entomol. 12: 929-932.

Logan, J. A., R. A. Casagrande and A. M. Liebhold. 1991. Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. Environ. Entomol. 20: 1516-1525.

Lyons, D. B. and T. J. Lysyk. 1989. Development and phenology of eggs of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), in Ontario, pp. 351-65. *In* Wallner, W.E. and K.A. McManus [eds.], Lymantriidae: a comparison of features of new and old world tussock moths. USDA Gen. Tech. Rep. NE-123.

Masaki, S. 1956. The effects of temperature on the termination of diapause in the egg of *Lymantria dispar* Linne. Jpn. J. Appl. Zool. 21: 148-157.

Mastro, V. C., T. M. Odell and C. P. Schwalbe. 1989. Genetic control of Lymantriidae: prospects for gypsy moth control. *In* W. E. Wallner and K. A. McManus [eds.], Lymantriidae: a comparison of features of New and Old World tussock moths. USDA For. Serv. Gen. Tech. Rep. NE-123. 554 pp.

Regniere, J. 1984. A method of describing and using variability in development rates for the simulation of insect phenology. Can Entomol. 116: 1367-1376.

Regniere, J. 1987. Temperature-dependent development of eggs and larvae of *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) and simulation of its seasonal history. Can. Entomol. 119: 717-728.

Regniere, J. 1990. Diapause termination and changes in thermal responsed during postdiapause development in larvae of the spruce budworm, *Choristoneura fumiferana*. J. Insect Physiol. 36(10): 727-735.

SAS Institute. 1990. SAS/STAT user's guide. Ver. 6. Vol. 2. SAS Institute, Cary, NC.

Sawyer. A. J., M. J. Tauber, C. A. Tauber and J. R. Ruberson. 1993. Gypsy moth (Lepidoptera: Lymantriidae) egg development: a simulation analysis of laboratory and field data. Ecological Modelling 66:121-155.

Schaub, L. P., F. W. Ravlin, D. R. Gray, and J. A. Logan. 1994. A landscape-wide model for predicting gyspy moth (Lepidoptera: Lymantriidae) phenology. Environ. Entomol. (submitted).

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal adaptations of insects. Oxford Univ. Press. 411 pp.

Tauber, M. J., C. A. Tauber, J. R. Ruberson, A. J. Tauber and L. P. Abrahamson. 1990. Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. Ann. Entomol. Soc. Am. 83(3): 494-503.

Waggoner, P. E. 1984. The hatching of gypsy moth eggs, a phenological model. Agric. Forest Meteorol. 33: 53-65.

Zar, J. H. 1984. Biostatistical Analysis. Prentice Hall. New Jersey. 718 pp.

Chapter 6

Summary

The need to maintain an appropriate relationship between stage of life cycle and season is of basic importance to insects living in seasonal climates. For example, egg hatch and larval feeding must coincide with the occurrence of adequate food. The phenology of the species is the set of adaptations that promotes appropriate timing of recurring biological events with annual cycles of the environment (Tauber et al. 1986). The processes of egg development are critical components of gypsy moth phenology. Gypsy moths spend almost nine months of the year as eggs. Winter survival is promoted by the cold hardiness of diapause. The events of postdiapause function in such a way that eggs to not hatch in response to a warm day in early spring. But an equally warm day later in the spring does promote a flush of egg hatch. Given this ability to appropriately time this critical event under various conditions, it is perhaps not surprising that the mechanism that governs egg development and hatch is complex. Despite many studies investigating the effect of temperature on gypsy moth egg development, there still does not exist a geographically robust, and validated, model of the process.

In conventional experiments investigating insect 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. 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. Phenology models of egg hatch have avoided the difficulty associated with a changing developmental response to temperature by assuming that the prediapause and diapause

phases are completed by an arbitrarily chosen calendar date and that only postdiapause remains to be completed.

In order to incorporate the changing developmental response into models of egg development, researchers have divided the egg stage into phases. In the model of Tauber et al. (1990), further developed by Sawyer et al. (1993), there is no clear transition between diapause and postdiapause. It is assumed that diapause begins on September 1 and thereafter the temperature-dependent developmental response gradually increases with physiological age. The gradually changing response is simulated by incorporating 200 maturity classes (or phases) into the developmental period from diapause to hatch.

Presented in the preceding chapters are data of respiration rates that can be interpreted as direct evidence of the multi-phase ontegeny of gypsy moth eggs. Immediately following oviposition, eggs begin the prediapause phase. This phase is characterized by high metabolic rates and morphological development of the embryo (Bell 1989). Measurements of respiration rate of individual eggs (Chapter 3) indicate that this phase can be completed, and diapause can be entered, without any environmental stimuli such as decreasing temperatures. These same measurements indicate that developmental rate during this phase is strongly temperature-dependent. Prediapause can be completed in 13 days at 31°C.

During prediapause, relatively vast quantities of stored triglycerides are depleted. Like developmental rate, the rate of triglyceride depletion is also strongly temperature-dependent (Chapter 4). The interaction of a temperature-dependent developmental time ($rate^{-1}$) and a temperature-dependent depletion rate would result in minimal total depletion occuring in those individuals that developed at approximately 25°C. Increasing volumes would be depleted by those individuals that developed at temperatures either higher or lower than 25°C. However, individuals with an inherently faster

developmental rate (independent of temperature) did not always deplete their triglyceride reserves faster. This lack of correlation between developmental rate and depletion rate contributed to a relatively uniform volume of triglycerides being depleted at all temperatures. This suggests that prediapause is completed when a required amount of embryological development has occurred, and that triglycerides are an important metabolic source for this development.

The effect of temperature on respiration rate remains relatively uniform throughout the phases of egg development (Chapters 3, 5). A 10°C decrease in temperature results in a 0.4 fold decrease in respiration rate, regardless of phase. However the pattern of respiration rate at any given temperature changes dramatically with developmental phase. Mean respiration rate at 25°C increases dramatically after 100 days at 5°C, indicating a phase transition.

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 (as per Sawyer et al. 1993). 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 1988).

Using the method described in Chapter 5 it is possible to identify critical patterns in respiration rate of individual eggs. These patterns can be used to distinguish the developmental phases. The data presented in Chapter 2 indicate a distinct transition between the prediapause and diapause phases. The identification of diapause entry permitted the estimation of a phase-specific developmental response to temperature for prediapause. The data presented in Chapter 5 indicate a distinct transition between the diapause and postpause phases. The increasing respiration rate during the postdiapause phase suggested that the developmental response to temperature may not be uniform through the phase. Additional data presented in Chapter 5 supported this hypothesis and a postdiapause-specific age-dependent developmental response to temperature was estimated. The functional form of this response (Fig. 5.7) describes an initially weak response to increasing temperature that is replaced with a stronger response as age in postdiapause increases. This response would have the effect of diminishing the likelihood that warm days in late winter or early spring result in egg hatch. However, response to the same temperature later in the spring has a greater effect since eggs are more physiologically advanced, and eggs will hatch. This effect is in accordance with field observations that egg hatch does not occur in early spring despite temperatures that are often as warm as those when egg hatch does occur.

A geographically robust model of gypsy moth egg development still has not been developed and independently validated. Although it has been demonstrated that egg ontogeny can be divided into three distinct phases on the basis of respiration rate, and phase-specific developmental responses to temperature have been estimated for prediapause and postdiapause, the precise temperature-time requirements of the diapause phase have not been adequately investigated. The small but significant increase in respiration rate at 25°C observed from day 1 to 100 (from onset of diapause) (Fig. 5.1),

together with the existence of the age-dependent developmental response to temperature estimated for postdiapause development (Fig. 5.7), suggest that an investigation of the developmental response to temperature in diapause include the capability of discerning an age-dependent influence.

Literature Cited

Bell, R. A. 1989. Respiratory activity during embryonic development in a diapausing and a selected non-diapausing strain of the gypsy moth, *Lymantria dispar* L. Comp. Biochem. and Physiol. 93A: 761-771.

Sawyer. A. J., M. J. Tauber, C. A. Tauber and J. R. Ruberson. 1993. Gypsy moth (Lepidoptera: Lymantriidae) egg development: a simulation analysis of laboratory and field data. Ecological Modelling 66:121-155.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal adaptations of insects. Oxford Univ. Press. 411 pp.

Tauber, M. J., C. A. Tauber, J. R. Ruberson, A. J. Tauber and L. P. Abrahamson. 1990. Dormancy in *Lymantria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. Ann. Entomol. Soc. Am. 83(3): 494-503.

VITA

David Richard Gray was born in Vancouver, Canada on March 7, 1952. He graduated from Hillside Secondary in 1970. After extensive travels in Europe, Africa, Asia, Central America and South America he began his university studies in 1977 and obtained a Bachelor of Science degree from Simon Fraser University in 1980. While working as a Regional Forest Entomologist for the British Columbia Ministry of Forest he obtained a Master of Pest Management degree in 1985 from Simon Fraser University under the supervision of Dr. John H. Borden. The title of his thesis was "Ambrosia beetles in a Vancouver Island dryland sort: their damage and proposed control". He entered the doctoral program at Virginia Polytechnic Institute and State University in September, 1988. Under the supervision of Dr. F. William Ravlin he investigated the phenological events leading to egg hatch of the gypsy moth.