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Early larval dispersal of gypsy moth (Lymantria dispar L.) : effects of maternal nutrition, provisioning of yolk proteins, and temperature during the egg stage.

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EARLY LARVAL DISPERSAL OF GYPSY MOTH (*LYMANTRIA DISPAR* L.): EFFECTS OF MATERNAL NUTRITION, PROVISIONING OF YOLK PROTEINS, AND TEMPERATURE DURING THE EGG STAGE

A Dissertation Presented

by

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To my parents, Sylvia and Charles Diss

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ABSTRACT

EARLY LARVAL DISPERSAL OF GYPSY MOTH (*LYMANTRIA DISPAR* L.): EFFECTS OF MATERNAL NUTRITION, PROVISIONING OF YOLK PROTEINS, AND TEMPERATURE DURING THE EGG STAGE

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North American gypsy moth disperse as larvae on the wind in a behavior called ballooning prior to feeding for the first time. Resources used in dispersal are therefore limited to those carried over from the egg.

I measured levels of two yolk storage proteins, vitellin (Vt) and glycine-rich protein (GRP), using quantitative immunoelectrophoresis. I determined the tendency of larvae to balloon in a wind tunnel. I estimated the length of the window for dispersal from the longevity of unfed neonates.

Pre-hatch levels of Vt and GRP had no influence on the tendency of neonates to balloon. Levels of these

proteins were positively associated with and accounted for 40-50% of the variation in longevity of neonates from the first-laid and center thirds of egg masses. Longevity was greatest for neonates from the first-laid third which also had the highest pre-hatch levels of Vt and GRP.

Nutritionally stressed females compensated to maintain levels of Vt and GRP by reducing the number of eggs produced. This compensation was reflected in similar longevities of offspring of stressed and unstressed females. The tendency of larvae to balloon, however, was greater in offspring of unstressed females. It is possible that traits selected for in nutritionally stressed females may be expressed in offspring as a reduced tendency to disperse.

Temperature during the six weeks prior to eclosion had a significant effect on pre-hatch levels of Vt and GRP and on neonate longevity. Eggs held at 7°C or less had similar pre-hatch levels of both proteins. Eggs held at 10°C for six weeks, however, were depleted of Vt and GRP suggesting a threshold between 7 and 10°C for stimulation of protein utilization. Eggs held for alternating weeks at 4 and 10°C had protein levels similar to eggs held at 4°C indicating eggs must be exposed to temperatures above the threshold for a period greater than a week before utilization of proteins is increased.

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CHAPTER 1

INTRODUCTORY COMMENTS

In the 126 years since its introduction, the gypsy moth, (*Lymantria dispar* L), has become established only in the northeastern quarter of the United States and adjoining areas of Canada, despite its ability to thrive on over 600 species of woody plants and wide climatic tolerance. This relatively slow spread is usually attributed to the inability of females to fly, and while larvae disperse by ballooning on silk, the majority move less than 150 meters (Liebhold and McManus 1991, Mason and McManus 1981). Dispersing even short distances, however, can enable larvae to escape hatching sites unsuitable for larval survival (Leonard 1970, Lance and Barbosa 1981, Hunter and Leckowicz 1992).

Because larvae balloon before feeding, some researchers have speculated that nutritional reserves carried over from the egg may influence dispersal (Leonard 1971, Capinera and Barbosa 1977, Leonard and Kunkel 1987). Previous work addressing the tendency to disperse measured activity of neonates (Leonard 1970, 1971, Barbosa et al. 1981) or the number that dropped on silk (Capinera and Barbosa 1976, Lance and Barbosa 1981). I thought larval dispersal could be better tested by measuring ballooning behavior in a wind tunnel. I also estimated the window

for dispersal from longevity of unfed neonates. Earlier studies of the effect of egg resources on dispersal used egg size as their criterion, a measure I felt was imprecise. I instead examined the effect on neonate longevity and dispersal of two yolk storage proteins that are present up to hatch: vitellin (Vt) and glycine-rich protein (GRP). I was able to quantify these proteins using antibodies developed by a colleague (Dompenceil 1992).

If egg resources play a role in determining dispersal of larvae, factors which influence the level of these resources are also likely to have an effect on dispersal. I investigated three such factors: 1) the nutritional experience of the maternal parent, 2) the distribution of Vt, GRP and egg weight among eggs in a mass, and 3) the temperature to which eggs are exposed.

To obtain results relevant to natural populations, I examined the influence of the nutritional experience of maternal parents by comparing offspring of females from completely (>95%) and lightly (10-20%) defoliated sites. Females from heavily defoliated sites are typically smaller and produce fewer eggs (Campbell 1978), reflecting the partial starvation they experienced as larvae (Barbosa et al. 1981). I compared levels of Vt and GRP and weight of eggs from the two defoliation levels following oviposition and just prior to hatch. I subsequently

tested neonates for longevity and tendency to balloon. I also investigated whether nutritional stress associated with defoliation affected levels of two hemolymph proteins in female prepupae and whether these proteins could be used to predict levels of Vt and GRP in their eggs. I examined levels of aryphorin (Ap), the most abundant hemolymph protein, and vitellogenin (Vg), the precursor of the yolk protein Vt using antibodies developed by my colleages, Sharon Karpells (1990) and Raquel Dompenciel (1992).

It has been suggested that differential provisioning of eggs by females could result in variation in the dispersal behavior of offspring (Dompenciel 1992, Barbosa et al. 1981). Size of eggs decreases in a gypsy moth egg mass along the order of oviposition (Leonard 1970); recent work with eggs dissected from females indicates that protein levels also change along the ovariole (Dompenceil 1992). I tested whether eggs from the first-, center, and last-laid thirds of the egg mass differed in weight or levels of Vt or GRP at the completion of oviposition as well as just prior to hatching. I then compared the longevity and percentage of neonates that ballooned from these sections.

The level of resources available to neonates is the product of the amount initially provisioned by the female minus the amount used during the egg stage. Dompenceil

(1992) found that about one-third of the amounts of Vt and GRP remain in the egg until hatching. I addressed the question of whether external conditions during the egg stage influenced the amount of these proteins available for neonates. I focused on temperature in the three months following oviposition and the period during late winter/early spring when pharate larvae become more responsive to external conditions (Gray 1994). For each temperature regime, I periodically measured levels of Vt and GRP. I also recorded and compared timing of hatch, neonate longevity, and ballooning for the egg temperature treatments.

The objectives of my studies should provide a better understanding of factors affecting larval dispersal in the gypsy moth.

CHAPTER 2

EFFECTS OF MATERNAL NUTRITION AND EGG PROVISIONING ON PARAMENTERS OF LARVAL HATCH, SURVIVAL AND DISPERSAL IN THE GYPSY MOTH, *LYMANTRIA DISPAR* L.

Abstract

North American gypsy moth disperse as newly hatched larvae on wind currents in a behavior called ballooning. Because ballooning occurs before neonates begin to feed, resources used in dispersal are limited to those carried over from the egg. I show that nutritional experience of the maternal parent can influence the tendency of offspring to disperse, and that resource provisioning of eggs by the maternal parent affects the duration of the window for dispersal. Offspring of females from defoliated sites had a lower tendency to balloon in a wind tunnel than larvae from females which had not experienced nutritional stress associated with host defoliation. The number of eggs in an egg mass, a reflection of the maternal parent's nutritional experience, also contributed to the predictive model for dispersal that included defoliation level. Egg weight and the levels of two yolk proteins, vitellin (Vt) and glycine-rich protein (GRP), however, had no influence on the

proportion of ballooning larvae. The length of survival without food, and thus the maximum period of time for dispersal, was correlated with levels of Vt and GRP, but not with egg weight. The level of defoliation at the site from which the maternal parent was collected was not related to the longevity of offspring, nor did it have a significant effect on the levels of Vt, GRP or egg weight. Levels of hemolymph proteins arylphorin (Ap) and vitellogenin (Vg) in the maternal parent during the prepupal stage had no influence on levels of yolk proteins, larval longevity, or tendency to balloon.

Introduction

The gypsy moth, *Lymantria dispar* (L.), is the most important forest defoliator in northeastern North America. Since its introduction to the Boston area in 1869 (Forbush and Fernald 1896), the gypsy moth has spread north into Canada, south to Virginia, and west to Michigan. The rate of range expansion of gypsy moth has been relatively slow for an insect with such wide host and climatic tolerances. Such slow expansion is understandable, however, when one considers that North American gypsy moth females are flightless and do not contribute to dispersal. While accidental transport of egg masses has played a role in colonization of new areas, dispersal on the wind by first

instar larvae is still the primary means of spread (Leibhold and McManus 1991, Elkinton and Liebhold 1990, Cameron et al. 1979, Leonard 1971a).

Dispersal takes place by a behavior called ballooning, where recently hatched larvae are borne aloft on silk threads. Once aloft, the direction and distance larvae are carried is dependent on wind conditions. Nearly all larvae settle within 120 meters of their takeoff point (Mason and McManus 1981); however, some can be transported many kilometers (Collins 1917, Nichols 1961). Larvae can have multiple episodes of ballooning (Capinera and Barbosa 1976), and such behavior would be advantageous where larvae settle onto an unfavorable location or host.

The period or window during which ballooning occurs is limited to the period between eclosion and first feeding (Leonard 1970, 1971a). Thus, the energetic requirements for ballooning must be met from the nutritional reserves carried over from the egg. It has been suggested that the nutritional experience and partitioning of resources among eggs by the maternal parent could influence the dispersal and survival of offspring (Leonard 1971b, Capinera and Barbosa 1977, Leonard and Kunkel 1988, Dompenciel 1992).

Studies of the influence of nutritional experience of the maternal parent on egg quality of gypsy moth have examined the effects of both quantity and quality of food.

The amount of food available to the maternal parent influences protein content and weight of eggs (Rossiter et al. 1988, Leonard pers. comm.), but not egg size (Barbosa et al. 1981, Capinera and Barbosa 1977). Tree species fed on by the maternal parent as a larva has a significant affect on egg size (Barbosa et al. 1981, Capinera and Barbosa 1977).

The relationship between quality of eggs and larval parameters relating to dispersal has also been examined. Eggs from dense populations are smaller and produce larvae that have a longer prefeeding stage and a higher level of activity (Leonard 1970, 1971b). Larvae from small eggs, however, are less oriented and slower to move towards light, and hang from silk threads less frequently than do larvae from large eggs (Barbosa et al. 1981, Capinera and Barbosa 1976, Lance and Barbosa 1981).

Rossiter (1991a, 1992) examined the interaction between the quality of the diet of the maternal parent and the length of the prefeeding stage of first instar larvae, a trait that is likely to be associated with the length of the window for dispersal. While she found that foliage quality had a significant influence on the length of time before offspring fed, she did not determine how the effect was transmitted from maternal parent to larvae.

The results of these studies suggest a link in gypsy moth between the nutritional experience of the maternal parent, partitioning of resources among eggs, and dispersal potential of offspring. No study has yet tested whether maternal nutrition influences provisioning of protein in eggs, subsequent tendency of larvae to balloon, and the length of the dispersal period. The development of antibodies specific to gypsy moth egg and hemolymph proteins (Karpells et al. 1990, Dompenciel 1992) allowed us to address these questions.

Storage proteins make up a significant proportion of the insect yolk (Kunkel and Nordin 1985). Vitellin (Vt) is the predominant protein in gypsy moth eggs (Dompenciel 1992) and is derived from vitellogenin (Vg), a hemolymph protein found in females. Vt is used during embryogenesis, but at least one third of the initial amount of Vt is present in eggs just prior to eclosion, indicating it could be a source of amino acids for neonates (Dompenciel 1992). Glycine-rich protein (GRP), another egg storage protein of gypsy moth, was described by Dompenciel (1992). Like Vt, about a third of the initial amount of GRP remains in eggs at the time of hatch.

I determined whether maternal nutritional experience affected levels of two serum storage proteins in the hemolymph of female larvae just prior to pupation. The

hemolymph proteins I focussed on were arylphorin (Ap), the predominent protein in the hemolymph (Karpells et al. 1990) and Vg, the precurser to Vt (Dompenceil 1992). I investigated whether levels of these hemolymph proteins could be used to predict levels of Vt and GRP in eggs just prior to hatch. I compared levels of Vt, GRP and egg weight between eggs from nutritionally stressed and unstressed females and examined whether maternal nutritional experience influenced the longevity of offspring and the percentage that ballooned. Finally, I considered whether levels of Vt and GRP or weight of eggs were related to longevity of first instars or their tendency to balloon.

Methods

Preparation of Antisera

I prepared a polyclonal antiserum against gypsy moth egg proteins including Vt and GRP using the method of Kunkel (1988). I dissected chorionated eggs from unmated females and homogenized 1 gm of eggs in 8 ml of PBS (0.15 M NaCl, 0.10M Na_2HPO_4 pH 7.2) and PMSF (phenylmethylsulfonyl fluoride). I centrifuged the mixture at 12,000 x g for 10 minutes at 4^{o}C and decanted the extract of yolk proteins. I then emulsified 1 ml of yolk protein extract 1:1 with complete Freund's adjuvant and injected it subcutaneously into a female goat. A month later, I gave the goat a

booster injection of the antigen with incomplete Freund's adjuvant. A week after the last injection, I prepared serum from the goat and measured the antibody titer. I bled the goat biweekly until the antibody titer fell below the level necessary to produce peaks that could be easily quantified.

Antibodies were precipitated with 0.5 vol. of saturated ammonium sulfate and resuspended in Buffer A (0.15 M NaCl, 0.10 M NaH_2PO_4, 0.05% EDTA, pH 7.0) containing 0.02% NaN_3. I separated the antiyolk serum into 5 ml aliquots and stored them at -20^{o}C until use. I titered Ap using polyclonal antiserum characterised previously (Karpells et al. 1990).

Protein Determination

I determined protein content of egg and hemolymph samples using quantitative or "rocket" immunoelectrophoresis (QIEP) (Laurell 1966), as modified by Kunkel (1988). Slides used for QIEP were produced by coating a 8 X 11 cm glass slide with 10 ml of a 1% agarose gel (Bio-Rad^{R} Standard low-Mr) containing a known percentage of the appropriate antiserum. I held slides prior to sample loading for no longer than three hours in a chamber at high humidity to minimize loss in volume from evaporation that could affect the height of precipitation peaks. I punched wells that held 3.3 ul of fluid sample in the gels immediately before loading them. I filled wells until the surface of the protein extract was flush with the edge of the well. Tests

comparing rocket heights of several samples of the same extract indicated that loading volume of samples was very consistent.

I combined samples of 5 eggs each with 500 ul 0.1 M Tris and 0.002 M TEC (ethylenedinitrilotetra-acetic acid (EDTA) adjusted to pH 8.6 with citrate) and homogenized them using an Omni^{R} 1000 microhomogenizer. I held egg homogenates on ice to prevent degradation of proteins, and loaded samples of the fluid portion into the gels within 15 minutes.

I took hemolymph samples immediately after collection of female prepupae from the field. Holding prepupae in a supine position, I pricked one proleg with a fine pin and collected two ul of hemolymph from the wound using a microcapillary tube. Prepupae recovered from the procedure with no apparent harm.

I prepared protein extracts of hemolymph immediately after hemolymph was collected. Two ul samples of hemolymph were expelled into individual 1.5 ml microcentrifuge tubes, and diluted with 198 ul of a 50/50 mix of gycerol and TEC saturated with phenythiourea to prevent melanization. I centrifuged samples at 6000 RPM for 10 min. at 4^{o}C to precipitate hemocytes and stored them at -20^{o}C for up to two weeks before assaying for Vg and Ap.

All slides included a sample of a standard so that sample results from different slides could be compared. The standard consisted of pooled hemolymph from female prepupae diluted to 50% with glycerol to prevent protein denaturation. Aliquots of standard were stored at -20^{o}C and diluted with TEC before use.

I electrophoresed loaded gels at 4^{o}C for 24 hours with a constant current of 6 mAmps per slide on a Bio-Rad^{R} Biophoresis$_{TM}$ horizontal electrophoresis cell with TEC as the conducting buffer. After electrophoresis, I rinsed slides with distilled water, pressed them under weights for 2 hours, then air-dried them. I stained gels overnight with 0.05% Coomassie blue R-250 in 7% acetic acid and then destained them with 7% acetic acid for 2 hours to highlight the zones of precipitation of the protein-antibody complex.

I identified the specific responses of the antiyolk serum to Vt and GRP using purified samples of these proteins. I measured the area under protein precipitation peaks, or "rockets" using a Bio-Rad^{R} Peak Height Area Estimator. I determined the amount of a protein in a sample by comparing the area under the rocket with values obtained from a series of dilutions of the purified protein.

The antiyolk serum was also used to quantify Vg, the hemolymph precurser of Vt. This serum produced a response to Vg when tested against a purified sample of this protein.

A similar response was produced against hemolymph samples, which contained Vg but not Vt. The cross reactivity of the polyclonal antiyolk serum to Vg is likely to be due to the similarity of molecular structure between Vt and Vg (Hagadorn and Kunkel, 1979).

Field Collection Sites

I collected egg masses and prepupae in Massachusetts and Vermont from sites with either high or low levels of defoliation. I assumed that larvae that matured at defoliated sites had experienced higher levels of nutritional stress. Populations I designated as highly stressed were from sites that were nearly or completely defoliated; populations with a low level of stress were collected from sites that sustained 10 to 20% defoliation of the canopy. Defoliation was visually estimated during the first week of July when larvae were in their last instar.

I sampled six populations each year, three from sites with high and three from sites with low levels of defoliation. Sites were separated by at least a kilometer. All collections were from boles of oaks in stands dominated by red, white, and/or chestnut oaks (*Quercus rubra*, *Q. alba*, and *Q. prinus*) (Table 2.1).

Collection and Handling of Prepupae and Eggs

After sampling for hemolymph, I placed prepupae in separate petri dishes at 24°C and a 16:8 hour photophase to pupate. I weighed female pupae three days after pupation. On eclosion, I placed each female in a paper bag with a laboratory reared male (standard lab strain NJSS) to mate and oviposit. I considered oviposition to be complete when the female moved off the egg mass, usually within 3 days of mating. I then marked the end of the mass where the last eggs were oviposited, clipped the egg mass out of the paper bag, and coded it to identify the maternal parent and collection site.

I also colleted egg masses from the field in late March from the same sites from which prepupae were collected the previous summer. I carved egg masses off oaks and marked the upper, tapered end, corresponding to the last-laid section of the mass. Masses were numbered and coded to indicate the collection site.

I placed eggs derived from field collected prepupae in an incubator at 24°C for 30 days, 20°C for 40 days, then 12°C for 20 days. Following this regime, I held eggs at 7°C until mid April. Egg masses collected from the field in late March were held at 7°C until testing.

Because of the large number of larvae used, I staggered hatching of egg masses over a three week period between the second week of April and the first week of

May. I randomly selected two to four masses daily to reduce confounding of treatment effects with potential differences associated with the length of time eggs were chilled. To promote hatch, I kept individual egg masses in petri dishes with a moistened dental wick at 20 ^{o}C, 16:8 hour photoperiod. Eggs were checked daily at 0900 and the neonates counted and removed. I calculated the total number of eggs for each mass from the number of larvae produced and the number of unhatched eggs.

Sampling of Eggs for Protein Analysis

Gypsy moths oviposit in consecutive order of development of eggs in the ovariole (Dompenciel 1992) and levels of some proteins change with the order of development in the ovariole (Dompenciel 1992, Diss unpubl.). For this reason, each egg mass was subsampled for Vt and GRP in three places. I took samples of 5 eggs each from the first-, center, and last-laid sections of each mass, then dehaired and weighed them when masses were moved from 7 to 20 ^{o}C to promote hatch. Eggs were stored at -20 ^{o}C, until testing (within a week). I calculated average levels of Vt and GRP for the mass from the values obtained from the three samples. Eclosion typically occurred within two to six days of sampling.

Test of Neonate Survival without Food

Each egg mass was represented by 60 larvae selected at random from those eclosing during the 3 days of peak hatch. Larvae were held 10 to a 15X100 mm petri dish with a moistened wick at 20^{o}C and 16:8 LD photoperiod. I checked larvae twice daily, at 0900 and 2100 and recorded the number of surviving larvae. Moribund neonates were gently prodded with a fine brush; those that did not respond I considered dead and removed them. Larvae that cannibalized and those fed upon were removed and subtracted from the total.

The duration of neonate survival without food was represented by the time until 50% of the subsample of 60 larvae of each mass remained alive. I determined the longevity of neonates from each mass by summing the total number surviving at each observation, plotting these values against time, and extrapolating from the sigmoid survival curve the time at which 50% of the initial population remained alive.

Test of Tendency of Neonates to Disperse

I tested the tendency of larvae to balloon in response to air movement in a wind tunnel with LxWxH of 120 X 25 X 25 cm. A squirrel cage fan was mounted on one end, and its speed was controlled by a rheostat. The air current from the fan passed through a 10 cm honeycomb

baffle to produce a laminar flow of air. Wind speed at differing settings of the reostat was calibrated using a hot-wire anemometer (Yokogawa, type 2141 JIS C 1102, class 1.5).

I exposed larvae on platforms of a 9 cm diameter card mounted on a 11 cm dowel at a 30^{o} angle to the horizontal. Two platforms were placed side by side, 15 cm from the front of the wind tunnel with the slope of the platforms angled up and away from the source of the air current, defecting it upwards. The room in which the wind tunnel was located was held at 20-21^{o}C and 40-60% RH. Lighting was from two ceiling mounted fixtures of two 4 ft, 34 watt, cool white, fluorescent bulbs.

I determined the tendency of larvae to balloon from a random sample of 60 neonates from each egg mass selected during the three days of peak hatch. After eclosion, I held neonates for two days 10 to a 15 X 100 mm petri dish with a moist wick at 20^{o}C and 16:8 photoperiod.

I tested larvae 48 hours after they had hatched. At 0900, I removed the wicks and placed larvae in the wind tunnel room to acclimate. I conducted tests between 1100 and 1500 hours, the peak period for ballooning in the field (Leonard 1971a). I placed 10 larvae on each of the two platforms in the wind tunnel to acclimate for two minutes in still air. The test took a total of six minutes. The fan was turned on and wind speed was

gradually increased to and maintained at 1.0 m/sec for two minutes, then 2.1 m/sec for two minutes. For the final two minutes of the test, I alternated the air speed between 1.0 and 2.1 m/sec in 15 second intervals. I recorded the number of larvae remaining on the platforms removed and discarded those in the wind tunnel. I represented the tendency for larvae to balloon as the percent of the total number of each egg mass that ballooned on silk from the platform.

Statistical Analysis

I used Analysis of Variance (ANOVA) to determine if the dependent variables of egg mass averages of egg weight, Vt and GRP content, number of eggs in the mass, ballooning, and longevity differed between collection sites. The mean square error term from this ANOVA, which represents the variation between egg masses within a site, was used in a t-test to determine if offspring of females from defoliating and nondefoliating populations differed in the variables mentioned above. I examined the influence of egg weight, protein levels, and the number of eggs in a mass on eclosion, larval dispersal and survival within a cohort using a stepwise regression procedure. The initial model consisted of a constant, the site of the maternal population, and level of defoliation at that site to separate out their influence. I used Pearson

correlations to determine relationships between egg weight, Vt and GRP content. Statistical calculations were done using BMDP (BMDP Statistical Software 1990) for mainframe computers.

Results

Factors Influencing Maternal Condition and Egg Provisioning

Pupal weights of females from defoliated sites and the number of eggs per mass they produced were significantly lower than those of females from undefoliated sites (Table 2.2). Variation in egg number was significantly greater among masses from defoliated sites ($P<0.01$, Bartlett's test of equal variances).

Both Vg and Ap levels were significantly lower in female prepupae collected from undefoliated sites ($P<0.01$ for both proteins). Variation in Vg and Ap levels was significantly greater among females from defoliated sites ($P<0.01$ for both proteins; 39 females from undefoliated and 139 from defoliated sites).

Although defoliation had a pronounced effect on female pupal weights and fecundity, it's effect on the egg characteristics measured was not consistent (Table 2.2). There was no significant influence on egg levels of Vt ($P=0.55$). There was also no consistent trend in Vt levels: in the 1990 cohort, eggs from defoliated sites had

higher mean Vt values, while in the 1991 cohort this pattern was reversed. Females from defoliated sites did tend to produce eggs with higher levels of GRP, whereas egg weight tended to be higher in eggs from sites which had experienced little defoliation (Table 2).

Factors Influencing Schedule of Eclosion

The effect of defoliation level on timing of eclosion was not consistent between the two cohorts. In the 1990 cohort, the length of time before the first eggs hatched after placement at 20^{o}C was greater among offspring of females from defoliated sites (3.7 days SE=0.2 vs 3.1 days SE=0.2 for undefoliated sites, P=0.03). The duration for 90% of eggs to hatch was similar in the two defoliation levels (2.2 days SE=0.1 for defoliated sites and 2.3 days SE=0.1 for undefoliated sites, P=0.75). In the 1991 cohort, all trends were reversed. Time to initiation of hatch was not different between defoliation levels, though the delay tended to be greater among eggs from undefoliated sites (5.0 days SE=0.2 for defoliated sites and 5.2 days SE=0.2 for undefoliated sites, P=0.32). Duration of hatch, however, was longer among eggs from defoliated sites (2.8 days SE=0.1 vs. 2.4 days SE=0.1 for undefoliated sites, P=0.01).

The average level of protein in eggs when they were placed at 20 ^{o}C was positively associated with the number

of days before the first eggs in the mass hatched. Both Vt and GRP appear to be important, though the association between these proteins and the length of delay in hatch was only significant in the 1991 cohort (1990 cohort: Vt $P=0.19$, GRP $P=0.10$. 1991 cohort: Vt $P<0.01$ $r^2=0.24$, GRP $P<0.01$ $r^2=0.19$).

The range in delay to initiation of hatching in masses from the two cohorts was very different. In the 1990 cohort, eclosion in all egg masses started within two to four days after being placed at 20 ^{o}C, and 70% had their first eggs hatch on day three. The range in delay of hatch was much greater in the 1991 cohort. The start of hatch varied between three and eight days after eggs were placed at 20 ^{o}C, and 80% of masses had their first eggs hatch within days four, five, and six.

The number of days for 90% of eggs in a mass to eclose was positively associated with the level of GRP ($P<0.01$, $r^2=0.13$) but not with Vt ($P=0.09$) in the 1991 cohort. Duration of hatch was two days for all but one egg mass in the 1990 cohort, and no association with level of yolk protein was apparent.

Factors Influencing Larval Survival

Food-deprived neonates of females from defoliated sites survived a mean of 139.7 hours (SE=5.04, N=55 egg masses) compared with 152.4 hours (SE=2.27, N=52 egg

masses) for offspring of unstressed females. The difference in longevity, however, was not significant (P=0.11) between defoliation levels. The site from which egg masses or their maternal parents were collected also had no significant influence on larval longevity (P=0.39 and 0.11 in 1990 and 1991 cohorts respectively). Neonate longevity was not correlated with the level of Vg (P=0.74) or Ap (P=0.47) in the prepupal hemolymph of the maternal parent in the 1990 cohort. Loss of eggs of the 1991 cohort due to an incubator malfunction prevented my repetition of this test.

The results of the stepped regression for the 1991 cohort showed Vt and GRP had a significant effect on neonate longevity (Table 2.3). The first random variable chosen for inclusion in the model by the program was GRP (P=0.002). With this variable in the model, Vt had no further significant effect, probably due to the high degree of correlation between the two proteins (Pearson correlation index=0.74). If GRP is excluded from the model, Vt will be entered (P=0.01). The model with defoliation level, site, and GRP accounts for 23% of the variation in longevity, with GRP accounting for 12% of that total. Figure 2.1 shows the positive relationship between neonate survival without food and the amount of Vt and GRP. Neither egg weight or the number of eggs in a mass had a significant effect on longevity in the 1991

cohort (Table 2.3). Results of the stepped regression of the smaller 1990 cohort indicated that neither Vt, GRP, egg weight, or the number of eggs in the mass made a significant addition to the model (Table 2.3).

Longevity was greater in the 1991 cohort than in the 1990 sample (P=0.02), although the mean length of survival only differed by approximately 8 hours. The amount of variation in longevity, however, was similar between the two cohorts (P=0.42).

Factors Influencing Larval Dispersal

Over both cohorts, a lower percentage of neonates from defoliated sites (21.8%, SE=1.8, N=52 egg masses) ballooned in the wind tunnel than did those from undefoliated sites (29.5%, SE=2.1, N=52 egg masses), (P=0.01). This difference between offspring of defoliating and nondefoliating populations was significant in both 1990 and 1991 cohorts (P<0.05, for each year). Site from which prepupae or eggs were collected had no significant influence on larval ballooning rates in either cohort (P=0.08 and 0.20 in 1990 and 1991 cohorts respectively).

There was no correlation between the maternal hemolymph levels of Vg (P=0.94) or Ap (P=0.48) and the percentage of offspring that ballooned in the 1990 cohort.

Loss of eggs due to an incubator malfunction prevented us from repeating this test with the 1991 cohort.

The stepped regression of data from the 1991 cohort showed the number of eggs in a mass made a significant contribution to the predictive model of the tendency of neonates to balloon (Table 2.4). This model, which included defoliation level and site as well as the number of eggs/mass, accounted for 19% of the variation in dispersal and number of eggs/mass explained 9% of this total. With eggs/mass in the model, Vt, GRP, and egg weight were not significant. Neither Vt, GRP, egg weight, nor the number of eggs/mass had a significant effect on tendency of neonates to balloon in the 1990 cohort (Table 2.4).

The average rate of ballooning was similar in 1990 and 1991 cohorts (P=0.47). Variability in the level of ballooning within each cohort was also similar between the two years (P=0.11).

Discussion

Results of earlier studies suggest that nutritional experience of the maternal parent might affect the likelyhood of offspring to disperse and that this influence could be exerted through the level of resources provisioned in the egg (Leonard 1970, 1971b, Barbosa et

al. 1981, Capinera and Barbosa 1976, Lance and Barbosa 1981, Rossiter 1991a, 1992). My results indicate that maternal nutritional experience and the level of proteins Vt and GRP in eggs affect the probability of dispersal through their influence on the tendency of larvae to balloon and the length of the window for dispersal. The effects of maternal experience and egg provisioning, however, are subtle and are not directly linked.

The nutritional stress experienced by females from defoliated sites was indicated by low female pupal weights and small numbers of eggs produced (Table 2.2). Partial starvation has been shown to result in decreased fecundity (Barbosa et al. 1981). Food deprivation as host trees are defoliated is likely a common source of nutritional stress among larvae in high density populations. It is important to consider that adult gypsy moths cannot feed, so resources used in the production of eggs must be accumulated in the larval stage.

I was surprised to find prepupae from nutritionally stressed populations had higher levels of hemolymph proteins Vg and Ap ($P<0.01$). It is difficult to interpret the significance of these results; levels of these proteins change rapidly during the prepupal stage, making timing of samples from field populations problematic. Ap increases during the prepupal period, then drops just prior to pupation (Karpells 1990). The level of Vg during

the prepupal period is also dynamic, and synthesis of Vg has recently been found to continue in the pupal stage (Dompenciel pers. comm.). Whether nutritional stress or genetic factors affect the timing of production or utilization of these proteins is not known, though I have observed variation in the timing of initiation of Vt synthesis. If such individual variation exists, comparison of samples taken at any one point during the prepupal stage may not be indicative of the protein resources that will eventually be available for incorporation into eggs.

Interestingly, while average weight of eggs was higher in masses from sites with low defoliation levels, this difference was not reflected in the levels of Vt or GRP (Table 2.2). There was no difference in the average level or degree of variation of Vt in eggs from defoliated and undefoliated sites. Thus, it seems nutritionally stressed females compensate for limited resources by producing fewer eggs, while maintaining normal levels of Vt. Compensation to maintain a minimum level of nutrient investment in individual offspring has been previously observed in other species (Slansky and Rodriguez 1987 and references therein). My data are the first, to my knowledge, of compensation to maintain levels of proteins in eggs from natural populations.

Surprisingly, GRP levels were higher in eggs from defoliated sites (Table 2.2). Levels of GRP also tend to be higher in the last-laid eggs of a mass, contrasting with reduced egg weight and lower levels of Vt (Dompenciel 1992, Diss 1996). It is possible that GRP may be used as a substitute for Vt among the last laid eggs, as utilization of both proteins occur at the same time.

The lack of covariance of weight, Vt and GRP levels in response to maternal stress shows that egg quality is not a uniform measure. Thus, egg size or weight are not necessarily good models for the distribution or amount of yolk proteins.

It is difficult to determine the significance of the correlation between higher levels of Vt and GRP and the number of days before the first eggs in a mass begin hatching. A difference of two or three days would seem to have little impact on larval survival unless synchrony of hatch with host phenology was disrupted. The effect of protein level may be more pronounced, however, under variable outdoor temperatures where the range in time to initiation of hatch is greater (Hunter and Lechowicz 1992).

Because dispersal by ballooning of larvae occurs almost exclusively before neonates feed (Leonard 1970, 1971a), the length of the prefeeding stage influences the potential for dispersal. I represented the maximum length

of the prefeeding period by the survival of neonates in the absence of food. Stress level of the maternal population as represented by level of defoliation does not have a significant influence on neonate longevity (P=0.11). Levels of Vt and GRP in eggs, however, are significant predictors of length of survival, at least among the larger 1991 cohort (Table 2.3, Fig 2.1). This result appears intuitive: the greater amount of resources carried over from the egg, the longer the larva could supply its metabolic needs without feeding. Egg weight, often considered a measure of egg resources, is not a significant predictor of longevity (Table 2.3). The association of Vt and GRP, but not egg weight, with survival indicates the fallacy of using general measures of egg quality such as weight or size as models for the action of specific egg resources.

The tendency to balloon is strongly influenced by the level of nutritional stress in the maternal population; offspring of stressed females have lower rates of ballooning. Also, the number of eggs in a mass, a reflection of nutritional experience (Campbell 1978, Barbosa et al. 1981), contributed to a predictive model for ballooning rates of offspring after defoliation level and site were included (Table 2.4).

How nutritional stress of the maternal parent is influencing the tendency of offspring to balloon is not

clear from my study. Average Vt and GRP levels in eggs prior to hatch were not correlated with dispersal rates of siblings. Direct comparison of Vt and GRP levels in larvae after wind tunnel tests was not possible since these proteins are utilized or altered so that the antibody was unable to recognize them within hours of hatch (Diss unpubl.). Average weight of eggs from a mass is not correlated with the tendency of larvae from that mass to balloon.

The lack of linear relationship between egg weight, Vt or GRP and tendency of larvae to balloon is particularly important in relation to other studies. There is some controversy on the role of egg quality in influencing dispersal of gypsy moth. Leonard (1970, 1971b), found smaller eggs from dense populations produce larvae with a longer prefeeding period and a higher activity level, even in the presence of suitable food, and suggests that larvae from smaller eggs are more likely to disperse. Others argue that larvae from small eggs are less likely to disperse because of reduced phototropism (Barbosa et al., 1981), and a lower tendency to descend on silk when acceptable hosts are available (Capinera and Barbosa 1976, Lance and Barbosa 1981). McManus and Mason (1983) hypothesize the predisposition of larvae to disperse is insignificant compared to the importance of environmental conditions in determining dispersal: a view

supported by Hunter's (1993) estimation that weather reduces a populations window for dispersal by an average of 54%. My findings that the tendency of offspring to disperse is associated with nutritional experience of the maternal population but not with egg protein or egg weight, suggest the presence of some other regulating factor internal to the insect. It is possible that the tendency to balloon is influenced by egg resources I did not measure, such as lipids. Alternatively, nutritional stress of females may select for traits which are expressed in neonates as a reduced tendency to balloon.

Conclusion

The potential for dispersal of gypsy moth is the product of the tendency of larvae to balloon and the length of the period in which dispersal can take place. Results of my study examining the influence of maternal nutritional experience and provisioning of protein in eggs on these two aspects of dispersal potential indicate that they are independently regulated. The length of survival without food, and thus probably the length of the prefeeding period in which dispersal occurs, was associated with the amount of Vt and GRP available to the neonate but not with the level of nutritional stress experienced by the maternal population. The fact that survival was not correlated with egg weight, an unspecific

measure of egg resources, should serve as a caution against assuming that general measurements of egg quality accurately model amount or action of specific nutrients. The tendency to disperse in neonates was associated with the level of nutritional stress in the maternal generation; however, this influence was not transmitted through yolk protein levels or resources associated with egg weight. Further research examining the role of other egg resources, such as lipids, in regulating the tendency of neonates to balloon may clarify the link between maternal nutritional experience and dispersal of offspring.

Table 2.1 Percent defoliation and tree species composition at each collection site. Sites within the Millers Falls township were separated by at least a kilometer.

Low Defoliation Sites

Year	defol.	Species composition of oaks	Locality
1990	20%	50% red, 50% chestnut	Miller's Falls, MA
1990	15%	60% red, 40% chestnut	Amherst, MA
1990	10%	40% red, 60% white	N. Hero Is., VT
1991	15%	50% red, 50% white	Belchertown, MA
1991	15%	50% red, 50% white	Hadley, MA
1991	20%	10% red, 90% white	Granby, MA

High Defoliation Sites

Year	defol.	Species composition of oaks	Locality
1990	95%	50% red, 50% white	Gill, MA
1990	90%	90% red, 10% white	Miller's Falls, MA
1990	99%	50% red, 50% white	Deerfield, MA
1991	95%	50% red, 50% white	Gill, MA
1991	90%	50% red, 50% chestnut	Miller's Falls, MA
1991	100%	100% red	Miller's Falls, MA

Table 2.2. Mean level of Vt, GRP, and weight of eggs, number of eggs produced, and pupal weights of female gypsy moths from defoliated and undefoliated sites. Standard error of mean follows it in parenthesis. Protein and weight values for egg masses are based on the average of three subsamples of five eggs each. N equals the number of egg masses from 1990 and 1991 cohort contributing to the values in the column.

Egg Character	Defoliated N=50	Undefoliated N=40	P
Vt (ug/egg)	21.5 (1.0)	23.1 (1.0)	0.55
GRP (ug/egg)	16.2 (1.0)	15.6 (0.4)	0.11
Egg wt. (mg/egg)	0.619 (0.07)	0.685 (0.05)	<0.01
Number of eggs per mass	115 (7.25)	345 (19.16)	<0.01
Pupal wt. (gm)*	0.704 (0.02)	1.245 (0.01)	<0.01

* calculated from 177 pupae from defoliated and 121 from undefoliated sites collected in 1991.

Table 2.3. Results of stepwise regressions of the longevity of food deprived neonates on average egg weight, Vt and GRP content, and number of eggs in a mass. Cohorts of 1990 and 1991 were analyzed separately. Site and defoliation level of the maternal population were incorporated into the model prior to the addition of the egg mass characters. N equals number of egg masses in a cohort.

	Probability level	
Character	1990 (N=19)	1991 (N=70)
Vt	P=0.57	P=0.01*
GRP	P=0.54	P<0.01, r^2=0.12
egg weight	P=0.97	P=0.31
number eggs	P=0.99	P=0.65

* Probability value before inclusion of GRP in the regression equation.

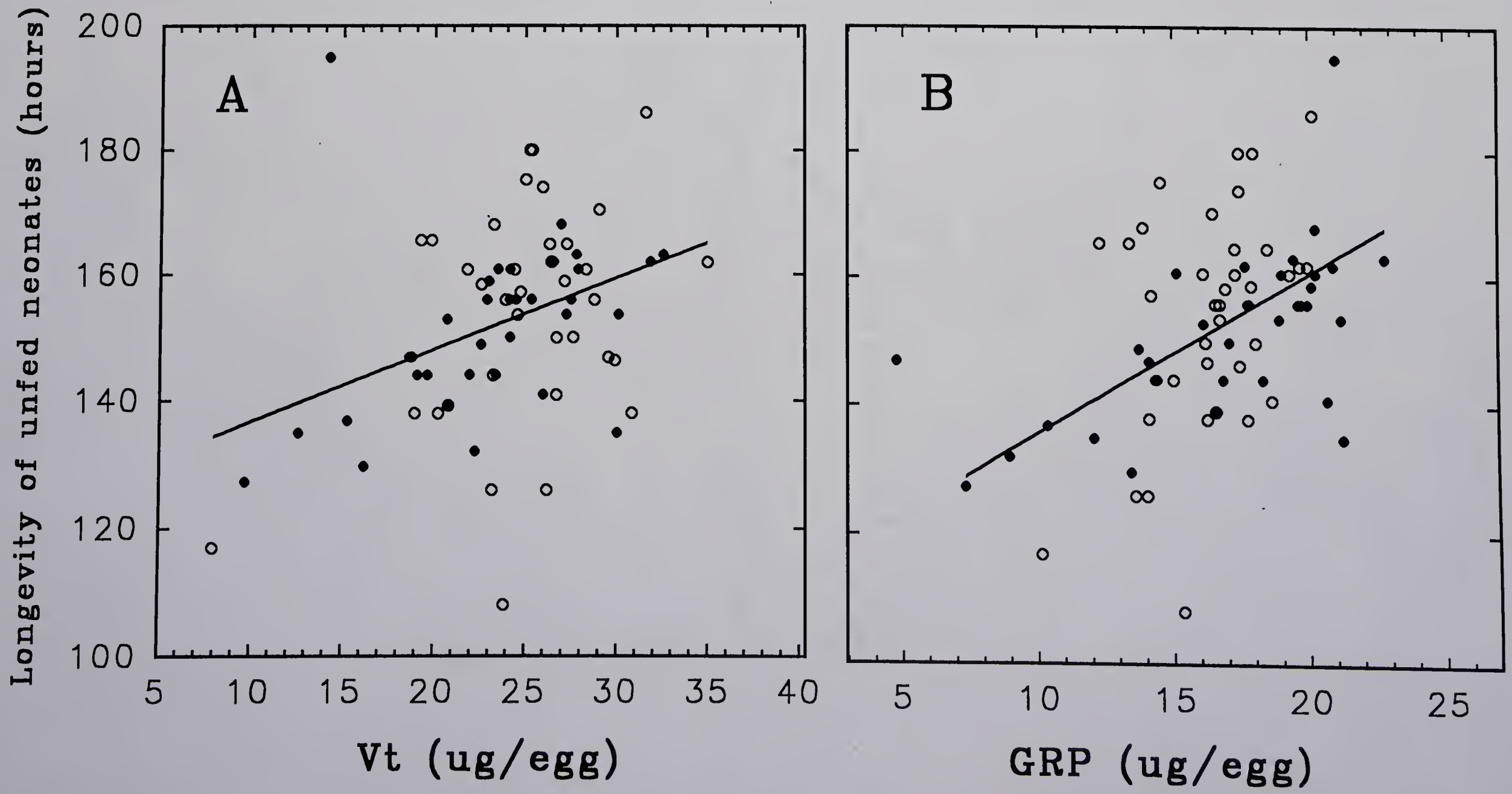
Table 2.4. Stepwise regressions of rate of ballooning on average egg weight, Vt and GRP content of egg masses. Cohorts of 1990 and 1991 were analyzed separately. Site and defoliation level of maternal population were incorporated into the model prior to the addition of the egg mass characters. N equals number of egg masses in cohort.

	Probability level	
Character	1990 (N=19)	1991 (N=70)
Vt	P=0.75	P=0.15
GRP	P=0.55	P=0.60
egg weight	P=0.54	P=0.53
number of eggs	P=0.09	P=0.01, r^2=0.09

Figure 2.1. Relationship between average levels of Vt (A) and GRP (B) in eggs of a mass and the longevity of unfed neonates from that mass. Fig A $P=0.04$, $r^2=0.06$, $n=70$. Fig B $P=0.006$, $r^2=0.10$, $n=70$. Data from 1991 cohort, values of these linear regressions differ slightly from those of the stepped regressions described in the text.

• masses from defoliated sites

○ masses from undefoliated sites



CHAPTER 3

EFFECTS OF EGG TEMPERATURES AND DEFOLIATION ON LEVELS OF YOLK PROTEINS AND DISPERSAL OF NEONATES IN THE GYPSY MOTH, *LYMANTRIA DISPAR* L.

Abstract

Higher temperatures during embryonation and early diapause did not affect levels of the proteins vitellin (Vt) or glycine-rich protein (GRP) in eggs of gypsy moth, *Lymantria dispar* L. This lack of response to temperature prevents depletion of these resources before diapause. During the six weeks prior to hatch, reduction in levels of Vt and GRP occurred in eggs maintained at 10°C, but not in eggs held at or below 7°C, or alternating weeks of 10 and 4°C. When placed at 20°C, larvae from eggs kept at 10°C eclosed sooner, suggesting an association of eclosion with the depletion of yolk proteins. Sensitivity to environmental temperatures during the early spring would facilitate synchrony of eclosion with bud expansion. Neonates from eggs held at 10°C for six weeks prior to hatch survived for a shorter period without food, possibly reflecting a shorter period for dispersal. The length of survival was correlated with the amount of Vt and GRP

prior to eclosion. Neither egg temperatures or levels of yolk protein, however, had an effect on subsequent tendency of larvae to disperse. Defoliation experienced by the maternal parent had no effect on egg weight, level of Vt or GRP in samples taken at the completion of oviposition, though pupal weight was reduced. Depletion of Vt and GRP in eggs during pre- and post-diapause periods were unaffected by maternal experience of defoliation.

Introduction

Yolk proteins are the primary nutrient resource in insect eggs (Kunkel and Nordin 1985). In the gypsy moth, *Lymantria dispar* L., vitellin (Vt) is the predominant protein in eggs and approximately one-third of the amount provisioned remains until eclosion (Dompenciel 1992). It has been proposed that the level of Vt in eggs just prior to hatch influences dispersal of neonates (Leonard 1970, Capinera et al. 1977, Leonard and Kunkel 1988), since dispersal occurs before larvae start to feed and thus the energetic requirements for dispersal must be met from nutritional reserves carried over from the egg.

Some of these authors have further speculated that higher than normal temperatures during the egg stage causes depletion of nutritional reserves, particularly Vt,

thus affecting disperal (Leonard 1970, Capinera et al. 1977). Isolation and production of antibodies against Vt and glycine-rich protein (GRP, another yolk protein present up to hatch) (Dompenciel 1992) allowed us to address this hypothesis. Here, I examine: 1) the influence of egg temperatures on levels of Vt and GRP during the pre- and post-diapause stages of egg development, and 2) the association of post-diapause egg temperatures with parameters of eclosion, neonate survival, and tendency of neonates to disperse in a wind tunnel.

Methods

General Methods

I collected gypsy moths at sites dominated by red oaks mixed with lesser amounts of white or chestnut oaks (*Quercus rubra*, *Q. alba*, and *Q. prinus*, respectively). Levels of defoliation were visually estimated at the time of pupation. To reduce variation associated with host species (Capinera and Barbosa 1977), I collected prepupae and eggs from boles of oaks.

Female prepupae were held individually at 24°C and a 16:8 LD photophase to pupate. On eclosion, I placed females in separate paper bags with a laboratory reared male (NJ strain). After oviposition was complete, I cut

egg masses from bags and marked the end of the mass containing the last-laid eggs. I labeled each mass to indicate the collection site of the maternal parent and the date of completion of oviposition.

I determined levels of the yolk proteins Vt and GRP in eggs using quantitative ("rocket") immunoelectrophoresis (QIEP) (Laurell 1966), as modified by Kunkel (1988). A description of the production of antisera, preparation of samples, and procedure for electrophoresis is found in Chapter 2.

I obtained initial of Vt, GRP, and egg weight for each egg mass from the average of three samples of five eggs each taken from the first, middle, and last oviposited thirds of the mass. In subsequent samples where egg masses were divided longitudinally, I determined levels of Vt and GRP and weight of eggs from one sample of five eggs selected along the long axis.

Influence of Defoliation on Egg Weight and Protein Levels in Newly Oviposited Eggs

I collected 12 to 19 female prepupae from each of six sites (a total of 86) and treated them as described above. Half of the prepupae were from three sites that were heavily defoliated (90-100%, sites H, I and J, Table 3.1), the remainder were from sites with light defoliation (15%, sites E, F and G). I weighed pupae the day after they

entered pupation, then replaced them at 24oC and 16:8 LD photophase to complete pupation. On the day a female completed oviposition, I took egg samples from the first-, center and last-laid sections as described above.

Influence of Temperature During the Fall on Egg Weight and Protein Levels

To determine the influence of temperature in the three months following oviposition, I collected 10 egg masses each from sites D (15% defoliated) and E (90% defoliated, Table 3.1). I then divided masses longitudinally in ca. equal halves to provide comparable test and control groups. I maintained one half of each mass under a control regime of 12:12 LD photophase and decreasing temperatures to approximate natural conditions during late summer and fall: 24^{o}C for 28 days, 20^{o}C for 42 days, and 12^{o}C for 21 days (a total of 91 days). I held the other half of each mass under the same time schedule, but temperatures at each step were 3^{o}C higher than the control temperatures: 27, 23 and 15^{o}C. I selected the 3^{o}C differential to approximate conditions of a warm autumn. I sampled eggs for protein analysis at each change in temperature and at the completion of the study on Day 92.

I also examined the effect of extending exposure to warm temperatures in the three months following oviposition. For this test I obtained 20 egg masses from

prepupae collected from an undefoliated site (F, Table 3.1). I took initial egg samples and divided masses longitudinally. I selected 10 egg masses randomly and held one half of each mass at 24°C for 28 days, 20°C for 42 days, and 12°C for 21 days, for a total test period of 91 days. The other half of these 10 masses I exposed to identical temperatures, but for seven days longer at the first (24°C) and second (20°C) steps. Temperatures were decreased on days 36 and 78 and the test period extended to 105 days. To determine if the response to extended exposure was similar at control and higher temperatures, I took the remaining 10 egg masses from the above site and maintained one half of each mass under the standard and one half in the extended schedule. The temperatures I exposed these egg to, however, were 3°C higher at each step of the two treatment regimes (27, 23 and 15°C). In all treatments, I took samples of five eggs each along the long axis of each half of the egg mass at each change in temperature and at the end of the test period, 92 or 106 days after oviposition.

Influence of Spring Temperatures on Egg Proteins and Neonate Eclosion, Survival and Dispersal

I examined the influence of temperature in the six weeks prior to eclosion on levels of Vt and GRP in two consectutive cohorts. In 1990, I collected female

prepupae from low-defoliation sites A and B (Table 3.1), and obtained egg masses as described above. Twenty-four egg masses, 12 from each site, were held under 12:12 LD photophase and a regime of decreasing temperatures, as described for the control treatment above, to approximate natural conditions. Thereafter, I kept masses for 129 days at 7°C and 8:16 LD photophase.

In mid-February, ca. 220 days after oviposition, I selected 12 masses, six from each site. I took initial egg samples as described above to determine mean levels of Vt and GRP. I then divided each mass longitudinally into thirds and randomly placed each third at 4, 7, or 10°C for six weeks. At the end of the treatment period, I selected five eggs from along the long axis of each section for protein analysis.

I used the remaining 12 egg masses from undefoliated sites A and B to test the influence of varying temperatures on protein levels. I sampled eggs for protein analysis in mid-February, as above, and divided masses longitudinally in half. I then held one half of each mass for six weeks at 7°C, and the other half under a regime of alternating weeks at 4 and 10°C. I sampled protein levels in eggs at the end of the six week period as described above.

I re-examined the influence of temperature in the post-diapause period the following year with 12 egg

masses, six from each of the low-defoliation sites E and F (Table 3.1), collected from the field in mid-February. I used treatments of constant 7 or 10°C, or outdoors (ambient).

To test whether nutritional experience of the maternal parent influenced utilization of Vt and GRP in eggs held at 7 or 10°C, I compared protein levels in eggs from two defoliated sites (I and J, Table 3.1) with those of similarly treated eggs from undefoliated sites (E and F, Table 3.1). I collected six masses from each site in mid February, sampled eggs for Vt and GRP, and divided masses longitudinally. I held one half of each mass at 7°C, the other at 10°C for six weeks, then sampled eggs again for Vt and GRP.

At the end of the six weeks of the various temperature treatments described above, I placed egg mass sections individually in 100x15 mm petri dishes with moist wicks at 20°C, 16:8 LD photoperiod to stimulate eclosion. I collected neonates daily at 0900 and recorded the number hatched. I noted the number of days before the first eggs of a mass hatched (initiation of hatch) and the period for 90% of the eggs from a mass to hatch (duration of hatch).

To determine if temperature in the post-diapause period influenced the longevity of neonates, I selected a random sample of 60 neonates from each egg mass section and placed them 10 to a 100x15 mm petri dish with a moist

wick at 20°C, 16:8 LD photoperiod. I checked larvae twice daily at 0900 and 2100, recorded the number of survivors and discarded those dead. In the few instances where I found a larva cannibalizing another, I removed both and deleted them from the data set. I continued observations until all larvae from a sample were dead. I plotted the number of survivors at each observation against time and extrapolated the time at which 50% of the sample survived.

Neonates from the test of 7, 10°C and outdoor temperatures were tested for tendency to disperse in a wind tunnel. Neonates disperse by a behavior called ballooning: larvae are borne aloft on wind currents with the help of a silk thread. I selected 60 neonates at random from each egg mass third, and kept them 10 to a 100x15 mm petri dish with a moist wick for 48 hours at 20°C and 16:8 photophase. At 0900 hrs, I moved larvae to the room containing the wind tunnel to acclimate for two hours. I began tests at 1100 and continued until 1500 hrs, the peak period for ballooning in the field (Leonard 1971). A complete description of the wind tunnel and test protocols is found in Chapter 2. In brief, the wind tunnel included a variable speed fan, a baffle to produce a laminar air flow, and two platforms located side by side. I placed 10 larvae on each platform, allowed them to settle for two minutes, then exposed them to wind velocities for six minutes in the following two minute

sequences: 1.0 m/sec, 2.1 m/sec, and alternating 1.0 and 2.1 m/sec in 15 sec intervals. I represented the tendency of larvae to disperse as the percentage that ballooned from the platform.

Influence of Overwintering Temperatures and Defoliation on Pre-hatch Levels of Egg Proteins, Neonate Eclosion and Dispersal

I obtained 32 egg masses from females collected as prepupae; eight from each of four sites. Two sites were heavily (90-95%, sites C and D), and two were lightly defoliated (15-20%, sites A and B, Table 3.1). I exposed eggs to the regime of 12:12 photophase and decreasing temperatures as described above for the fall temperature studies: 24°C for 28 days, 20°C for 42 days and 12°C for 21 days. I then placed egg masses at 7°C and 8:16 LD photophase until April. I revisited collection sites on March 28-30 and collected five egg masses at each. I marked the upper-most (last-laid) end of each egg mass and held them at 7°C and 8:16 LD photophase until they were tested in early April.

In April, I sampled five eggs each from the first-, center and last-laid sections of a mass for protein analysis and then placed masses in individual petri plates with a moist wick at 20°C and 16:8 photophase to stimulate eclosion. I collected and tested neonates for survival

and tendency to disperse as described above.

Unfortunately, I were unable to analyze the survival data. Egg masses from defoliated sites were small and many did not yield sufficient numbers of larvae for tests of both dispersal and longevity.

Results

Influence of Temperature in the Fall on Protein Levels and Egg Weight

Approximately two-thirds of the Vt and one-third of the GRP was utilized in the first 29 days (Fig 3.1A). After 29 days, there was little change in levels of Vt and GRP. Weight of eggs decreased rapidly in the first 29 days, with a lower rate of loss over the next 62 days (Fig. 3.1B).

The rate of weight loss during the 70 days following oviposition was greater in eggs held 3°C above the control ($P<0.01$ from linear contrast of slopes, Fig. 3.2). During the 91 days following oviposition, temperature did not influence the rates of decrease in levels of Vt ($P=0.10$) or GRP ($P=0.77$).

To determine the effect of prolonging the period of exposure for two weeks, I used linear contrast to compare the rates of decline in levels of Vt, GRP and egg weight between egg mass halves held for different periods. I

analyzed separately eggs held at control and temperatures 3°C higher because initial protein values indicated the two samples were not compatable. Prolonging the period of exposure for two weeks did not affect weight loss (control temperature, P=0.41; 3°C above control, P=0.42), though loss tended to be greater in eggs held at higher temperatures (Fig. 3.3). The rate of reduction of Vt or GRP for eggs in either temperature regime was also unaffected by prolongation of exposure (control temperature -- Vt P=0.83, GRP P=0.08; 3°C above control -- Vt P=0.90, GRP P=0.76).

Influence of Defoliation on Protein Levels and Egg Weight in the Fall

At the completion of oviposition, average levels of Vt, GRP, and egg weight were similar in egg masses from defoliated and undefoliated sites (Table 3.2). Eggs from site H (90% defoliated) and site F (15% defoliated) did not differ in initial levels of Vt or GRP or in egg weight (P=0.06, 0.26, and 1.00 respectively), though female pupal weights were lower (P<0.01). The rate of change of these characters during the first 91 days (Aug. - Oct.) was similar (Vt P=0.57, GRP P=0.55, egg weight P=0.74).

Influence of Spring Temperatures on Egg Proteins and Neonate Eclosion, Survival and Dispersal

Eggs held at 10°C for six weeks prior to sampling in April had lower levels of Vt and GRP prior to hatch than did eggs from the same masses held outdoors or at constant 4 or 7°C (Table 3.3). Eggs exposed to alternating weeks of 4 and 10°C had Vt and GRP levels similar to those of eggs held at constant 7°C.

Egg temperature during the six weeks prior to eclosion significantly affected the time to initiation of eclosion after eggs were placed at 20°C (Table 3.3). Eggs from the 10°C treatment hatched before those from the 4 and 7°C treatments. Eggs held at constant 7°C or alternating weeks at 4 and 10°C started hatching at about the same time. Eggs kept outdoors had a significantly longer delay before hatching than did eggs from the same egg masses held for six weeks at 7 or 10°C. Levels of Vt and GRP were positively associated with the length of time to initiation of eclosion ($P<0.01$ for both proteins in both cohorts), and accounted for one-half to two-thirds of the variation in this parameter (Fig. 3.4).

The length of time required for 90% eclosion was statistically similar among eggs previously held at 4, 7 or 10°C (Table 3.3). Eggs kept outdoors hatched over a longer period than did eggs held at 10°C. The duration

for 90% eclosion for eggs held outdoors, however, was not different from that of eggs kept at 7°C. Duration of eclosion within temperature treatments was not influenced by the level of defoliation experienced by the maternal parent (P=0.79, 0.78 for eggs held at 7 or 10°C, respectively). The duration for 90% eclosion increased with higher prehatch levels of Vt (P=0.01 in both cohorts) and GRP (P<0.01 in 1990, P=0.08 in 1991). Protein levels of eggs, however, accounted for only about 12% of the variation in duration of eclosion.

The longevity of unfed neonates from eggs held at 10°C was about half a day, or 10% less, than that for neonates from eggs held outdoors or maintained at constant 4 or 7°C. Neonates from eggs held at 7 or alternating 4 and 10°C survived for similar lengths of time (Table 3.3). Length of neonate survival was positively associated with pre-hatch levels of Vt (Fig. 3.5, P<0.01 for regressions in both cohorts) and GRP (P=0.03 and 0.05 in 1990 and 1991 cohorts, respectively). The amount of variation in longevity explained by these yolk proteins, however, was relatively low. Level of Vt accounted for 20 and 17%, and GRP 13 and 18%, of the variation in neonate longevity in 1990 and 1991 cohorts respectively.

Egg temperatures did not subsequently influence the proportion of neonates that ballooned in a wind tunnel (P=0.83 from a two-way ANOVA with temperature treatment

and egg mass as independent factors). From eggs held at 7, 10°C, or outdoors, the percent of larvae that ballooned was 18.8, 21.5, and 21.1% respectively. The level of Vt or GRP prior to hatch was not correlated with the rate of ballooning in a test of linear regression (Vt P=0.45, GRP P=0.19).

Influence of Defoliation and Spring Temperatures on Egg Proteins and Neonate Eclosion

Defoliation experienced by the maternal generation had no effect on levels of Vt or GRP following six weeks at 7 or 10°C in February and March (P=0.81 and 0.41 respectively, from a two-way ANOVA of defoliation and temperature), nor did the interaction of defoliation and temperature (P=0.49 and 0.55 respectively). As in the previous test, eggs held at 10°C had significantly lower levels of Vt and GRP than did eggs held at 7°C in the April sample of eggs. Time to eclosion following placement at 20°C was unaffected by defoliation (P=0.77) or the interaction of defoliation and temperature (P=1.00) but was significantly shorter among eggs held at 10°C (P<0.01).

Influence of Overwintering Temperatures and Defoliation on Pre-hatch Levels of Egg Proteins, Neonate Eclosion and Dispersal

Wintering treatment had a significant effect on pre-hatch levels of Vt and GRP (Table 3.4): eggs held outdoors under ambient conditions had higher levels of both proteins compared with eggs held under laboratory conditions (Table 3.5). The level of defoliation experienced by the maternal population did not affect levels of Vt and GRP prior to eclosion (Table 3.4). While differences in Vt and GRP between the two wintering treatments appeared to be greater among eggs from defoliated sites (Table 3.5), the interaction of defoliation and wintering treatment was not statistically significant.

I examined the percentage of larvae dispersing in the wind tunnel with a model of fixed and random factors (Table 6) and found the only significant predictors were defoliation level and the number of eggs per mass. Overwintering temperature treatments, egg weight, and levels of Vt and GRP showed no influence on neonate dispersal. A greater percentage of neonates from undefoliated sites ballooned than from defoliated sites (29.2 vs. 22.2% respectively), a result I had previously observed. By itself, fecundity had no relationship to neonate dispersal (r^2=0.01), but within the same

defoliation catagory, a greater percentage of neonates ballooned from small than from large egg masses. The mean fecundity for the undefoliated and defoliated sites in this test were 463 and 181 eggs/mass respectively.

Discussion

Initial egg weight and levels of Vt and GRP were similar in eggs from defoliated and undefoliated sites. The similarity of eggs is interesting because females from defoliated sites had lower pupal weights and smaller egg masses, two characteristics associated with nutritional stress in gypsy moths (Barbosa et al. 1981). I proposed in Chapter 2 that females were compensating to maintain an average level of Vt and GRP by reducing fecundity to explain the similarity in prehatch protein levels of eggs from sites defoliated or not defoliated by the maternal generation. That average protein levels and egg weight were also similar at completion of oviposition as I show here lends further strength to this explanation.

The reduction of Vt in the 28 days following oviposition and subsequent lack of change for the next 63 days in natural populations (Fig. 3.1a) is similar to the pattern described by Dompenciel (1992) in the New Jersey laboratory strain of gypsy moth. While the corresponding decrease in GRP is less than that of Vt, the pattern over

time is similar. The reduction in both proteins corresponds to the period of embryonation (Bell 1989).

Rates of depletion of Vt and GRP in the three months following oviposition were not affected by a 3°C difference in temperature. This is surprising since metabolic rate during embryonation is significantly higher with increases in temperatures of as little as 5°C (Gray et al. 1991). If Vt and GRP serve as energy sources for respiration, I would expect that amounts of these proteins utilized in the first 29 days would be greater at the higher temperature. Nevertheless, more than half of the Vt and GRP provisioned at oviposition is depleted during this period, suggesting that while these proteins may play a minor role in respiration, they may be an important source of amino acids for the formation of the pharate larvae.

While I found no effect of a 3°C difference in temperature on protein utilization, Capinera et al. (1977) showed a trend of higher levels of yolk protein in eggs held at 15 than at 27°C for 42 days following oviposition. After calculating and comparing confidence intervals for their values, however, I believe that the results of their two treatments are not significantly different.

The pattern of greatest loss of weight in eggs during the first 30 days (Fig. 3.1b), followed by a reduced, but sustained, decline may reflect changes in metabolic

activity. Gray et al. (1991) and Bell (1989) showed the rate of respiration in eggs of gypsy moth is greatest during the first 29 days and then decreases to a low level with the establishment of diapause.

Eggs at 27°C lost more weight in the first 28 days than those at 24°C (Fig. 3.2), likely reflecting the higher rate of respiration in response to increased temperature observed by Gray et al. (1991). Rates of weight loss in both temperature treatments decreased to a similar low level following day 30, as did respiration rates of eggs held at temperatures between 20 and 38°C following day 25 (Gray et al. 1991).

Extending the treatment period from 91 to 105 days did not influence levels of proteins or loss of weight at either control or elevated temperatures. While Gray et al. (1991) found duration of the prediapause stage decreased with increasing temperature, eggs in their treatments (ranging from 15 to 38°C) completed embryogenesis and had low levels of respiration by day 22. It seems unlikely, therefore, that I would see any differences in protein level given I took my first samples on days 28 and 36, after embryogenesis was complete. While weight loss continues after the formation of the embryo, the 14 day extension, from 91 to 105 days, may not have been sufficient for a measurable difference in egg weight.

Following diapause, temperature has a significant, though apparently non-linear, influence on depletion of Vt and GRP. Eggs held for six weeks at 7°C and 10°C retained 94 and 14%, respectively, of their February levels of Vt. Levels of Vt in eggs held at 4°C for six weeks were similar to those from the same egg mass held at 7°C. Utilization of GRP at the above temperatures followed a pattern similar to that of Vt. The large difference in amounts of protein utilized in eggs held at 10 and 7, vs. 7 and 4°C suggests a threshold exists between 7 and 10°C where utilization of Vt increases abruptly.

Levels of Vt and GRP tended to be higher in eggs held outdoors compared with those held at 7°C for six weeks in early Spring, but the difference is not significant (Table 3.3). This is a surprising result since protein levels in eggs held outdoors for the entire egg stage (Aug.-April) were significantly higher than those of eggs held under laboratory conditions (Table 3.4). Two factors may be acting to reduce the difference in response to the two temperature treatments in the early Spring study. The eggs used in the early Spring test were from undefoliated sites.

Results from the test run over the entire egg stage suggest eggs from undefoliated sites have a smaller disparity in protein utilization when held outdoors vs. 7°C (Table 3.4). The tests of response to temperature

over the entire egg stage and the test focusing on reponse during early Spring were run in consecutive years; differences in outdoor temperatures in these two years could have contributed to the lack of significance between treatments in the early Spring study.

Level of Vt and GRP prior to hatch was positively associated with the length of time until neonates started eclosing after eggs were placed at 20^{o}C (Fig.3.4). I had previously observed the same association among eggs collected from the field in April. It seems likely that high temperatures in Spring stimulate metabolic changes in preparation for eclosion that result in the depletion of yolk proteins.

The length of time eggs are exposed to a given temperature appears to be important in determining rate of depletion of Vt and GRP and time to eclosion. Exposure of eggs to 10^{o}C for six weeks resulted in significantly lower pre-hatch levels of Vt and GRP and earlier eclosion (Table 3.3). In contrast, eggs held at 4 or 10^{o}C on alternating weeks for six weeks had pre-hatch levels of Vt and GRP similar to those of eggs kept at 4^{o}C for six weeks. Eggs from both constant 4 and alternating 4 and 10^{o}C started hatching after 4 days at 20^{o}C, as compared with approximately 2 days for eggs held at 10^{o}C.

Less yolk protein may have been utilized in eggs held under alternating weeks at 4 and 10^{o}C because they spent

three rather than six weeks at 10°C. While a shorter period of exposure to 10°C may explain some of the difference in protein levels between treatments, it seems unlikely to account for the , however, given the 5 fold difference in Vt and 3 fold difference in GRP levels of eggs held under alternating 4 and 10°C vs. constant 10°C at the end of the treatment period (Table 3.3).

The requirement for higher temperatures to persist before protein levels decline could deter depletion of Vt and GRP and possibly prevent eclosion in response to brief warm periods in late winter. The length of exposure to a given temperature before metabolic changes associated with eclosion are stimulated (such as those resulting in depletion of Vt and GRP) is likely determined by the temperature and the number of days at or below 7°C already experienced. Gray et al. (1994) showed that metabolic rate in the post-diapause period increases in response to increased temperature, and that the amount of change in metabolism in response to a given temperature increases with age of the egg. Furthermore, older eggs respond more rapidly to an increase in temperature; Tauber et al. (1990) showed that the length of time to eclosion after placement at 21°C decreased in eggs taken from the field from Dec. to March. I also observed that moving eggs from 7 to 20°C stimulated eclosion after two to six days in

April, but in February, eclosion occurred after seven to 12 days (Diss, unpublished data).

I had previously observed a positive association of longevity of neonates with pre-hatch level of Vt among collections from field populations taken in April (Diss et al. in press). That longevity decreased in conjunction with pre-eclosion levels of Vt and GRP among eggs from the same mass held at 10°C vs. 7 or 4°C in the post-diapause period gives further support to the role of these yolk proteins in influencing neonate longevity. Prehatch levels of Vt and GRP, however, account for 20% or less of the variation in neonate longevity. This weak association is reflected in the relatively small 10 to 17 hour difference in longevity between neonates from eggs held at 10°C, which had nearly depleted reserves of Vt and GRP, and neonates from eggs from the other temperature treatments which had five to six times more Vt of eggs from the 10°C treatment. Furthermore, even neonates from egg masses with no detectable Vt or GRP survived for comparable periods (Fig. 3.5). While higher temperature during the post-diapause period decreases larval longevity, and therefore the period available for ballooning, the small difference in longevity may not have a substantive effect on rates of dispersal in field populations.

The association of the tendency to balloon with

the level of defoliation and fecundity suggest that nutritional stress of the maternal parent can influence the dispersal of offspring. A possible mechanism for translating this effect between generations is through resources provisioned in the eggs. Temperature, particularly during the post-diapause period, influences respiration (Gray et al. 1994, 1991). Increased metabolism in response to higher temperatures is probably the cause of the depletion of Vt and GRP I observed during the post-diapause period, and it seems likely that other egg resources were similarly reduced. The lack of association between temperature during the egg stage and ballooning, however, suggests that the maternal influence on this behavior is not being transferred through nutritional parameters.

Table 3.1. Percent defoliation and tree species composition at collection sites in Massachusetts. Sites within Millers Falls township were separated by at least a kilometer.

Site	Year	Defol.	Species composition of oaks	Locality
A	1990	20%	50% red, 50% chestnut	Millers Falls
B	1990	15%	60% red, 40% chestnut	Amherst
C	1990	95%	50% red, 50% white	Gill
D	1990	90%	90% red, 10% white	Millers Falls
E	1991	15%	50% red, 50% white	Hadley
F	1991	15%	50% red, 50% white	Belchertown
G	1991	15%	50% red, 50% white	Granby
H	1991	90%	50% red, 50% chestnut	Millers Falls
I	1991	100%	100% red	Millers Falls
J	1991	95%	50% red, 50% white	Gill

Table 3.2. Mean (SE) of Vt, GRP, and weight of eggs immediately following oviposition, and pupal weights of female gypsy moths from six sites: three >90% and three sites 10-20% defoliated. Each site contributed data from 12 to 19 egg masses to the means. N below the mean is the number of egg masses or pupae that contributed to it. Probability values for the effect of defoliation are from an ANOVA which included interactions of defoliation and site and that of defoliation, site and egg mass as error terms.

	Defoliated	Undefoliated	P
Vt (ug/egg)	87.3 (1.4)	89.8 (1.2)	0.43
	(N=40)	(N=41)	
GRP (ug/egg)	32.7 (0.7)	31.8 (0.9)	0.73
	(N=43)	(N=43)	
Egg wt. (mg/egg)	0.739 (0.01)	0.754 (0.01)	0.24
	(N=41)	(N=42)	
Pupal wt. (gm)	0.776 (0.02)	1.258 (0.03)	0.001
	(N=42)	(N=42)	

Table 3.3. Mean (SE) level of Vt and GRP prior to eclosion, number of days to initiation of hatch, duration until 90% of larvae eclosed, and hours survived by 50% of neonates from eggs held at several temperature regimes during the six weeks prior to hatch. Within tests, treatment means followed by different letters are significantly different at the 5% level as determined with a two way ANOVA with treatment and egg mass as independent factors or t-test. Twelve egg masses are represented in each treatment.

Table 3.3. Mean (SE) level of Vt and GRP prior to eclosion, number of days to initiation of hatch, duration until 90% of larvae eclosed, and hours survived by 50% of neonates from eggs held at several temperature regimes during the six weeks prior to hatch. Within tests, treatment means followed by different letters are significantly different at the 5% level as determined with a two way ANOVA with treatment and egg mass as independent factors or t-test. Twelve egg masses are represented in each treatment.

Treatment of eggs (°C)	Vt (ug/egg)	GRP (ug/egg)	Days to initiation of hatch	Duration of hatch (days)	Survival of neonates (hrs)
Test of Constant Temperatures					
4	23.5 (1.5)a	19.4 (0.8)a	4.1 (0.4)a	3.1 (0.2)a	134.5 (3.7)a
7	26.8 (1.1)a	21.4 (0.8)a	4.9 (0.2)a	3.3 (0.2)a	139.0 (2.9)a
10	4.3 (2.3)b	7.8 (2.4)b	1.8 (0.3)b	2.7 (0.1)a	123.3 (3.6)b
Test of Alternating High and Low Temperatures					
control (7)	24.8 (1.4)a	19.2 (0.8)a	4.3 (0.1)a	3.3 (0.2)a	140.7 (4.0)a
alternating 4 and 10	21.3 (1.5)a	18.0 (1.0)a	4.0 (0.0)a	2.4 (0.3)b	140.3 (3.3)a
Test of Ambient Outdoor vs Constant Temperatures					
ambient outdoor	29.2 (1.2)a	19.6 (0.4)a	10.3 (0.4)a	3.3 (0.2)a	169.4 (3.5)a
7	28.1 (0.8)a	18.2 (0.4)a	6.6 (0.3)b	3.1 (0.1)ab	174.8 (3.5)a
10	14.6 (1.5)b	12.2 (0.8)b	2.3 (0.2)c	2.8 (0.1)b	158.4 (3.9)b

Table 3.4. Results of ANOVA of the effects of defoliation, whether eggs were wintered under ambient outdoor temperatures or at 7°C and the interaction of these two factors on egg weight and levels of Vt and GRP prior to eclosion. The model included as error terms the interaction of defoliation and site and the interaction of defoliation, site, wintering condition and egg mass.

	Defoliation	Wintering treatment	Interaction
Vt	P=0.74	P=0.03	P=0.07
GRP	P=0.59	P=0.02	P=0.10
Wt.	P=0.58	P=0.12	P=0.75

Table 3.5. Mean Vt, GRP and weight of eggs from sites of high (90-95%) or low (15-20%) defoliation maintained until April outdoors under ambient temperatures or at 7°C. Means for ambient outdoors treatment are calculated from five egg masses from each site. Means for 7°C treated eggs are calculated from eight egg masses from each of sites B and D, nine from site A and seven from site B.

		Vt (ug/egg)			GRP (ug/egg)			Wt. (mg/egg)		
Defol.	Site	Amb.	7°C	Diff.	Amb.	7°C	Diff.	Amb.	7°C	Diff.
low	A	14.8	14.6	0.2	11.3	11.2	0.1	629	619	10
	B	17.2	16.7	0.5	13.9	12.7	1.2	680	637	43
high	C	18.0	14.7	3.3	15.6	12.2	3.4	646	605	41
	D	18.8	13.4	5.4	14.3	10.5	3.8	654	614	40

Table 3.6. Results of ANOVA of dispersal using complete and reduced models. Wintering treatments were outdoor ambient or 7°C in the laboratory. Thirteen egg masses were obtained from each of four sites: two heavily (90-95%) and two lightly (15-20%) defoliated. Eight egg masses from each site were held at 7°C and five were wintered outdoors under ambient temperatures.

	Complete model			Reduced model		
	DF	MS	P	DF	MS	P
Defoliation	1	755.64	0.01	1	1523.15	<0.01
Defol.*Site	2	76.00		2	28.60	
Winter	1	21.50	0.67			
Winter*Defol.	1	2.66	0.88			
Eggs/mass	1	621.24	0.03	1	900.04	0.01
Vt	1	54.44	0.50			
GRP	1	309.66	0.12			
Egg weight	1	17.9	0.70			
Residual	42			47		

r^2 of reduced model = 0.21

Equation for percent dispersal

% dispersal = 43.21 - 15.54(defol.= 0, 1) - 0.03(eggs/mass)

Figure 3.1. Changes in average levels of Vt, GRP (A) and egg weight (B) over the 91 days following oviposition. Eggs were maintained under a temperature regime of 24°C for 28 days, 20°C for 42 days, and 12°C for 21 days.

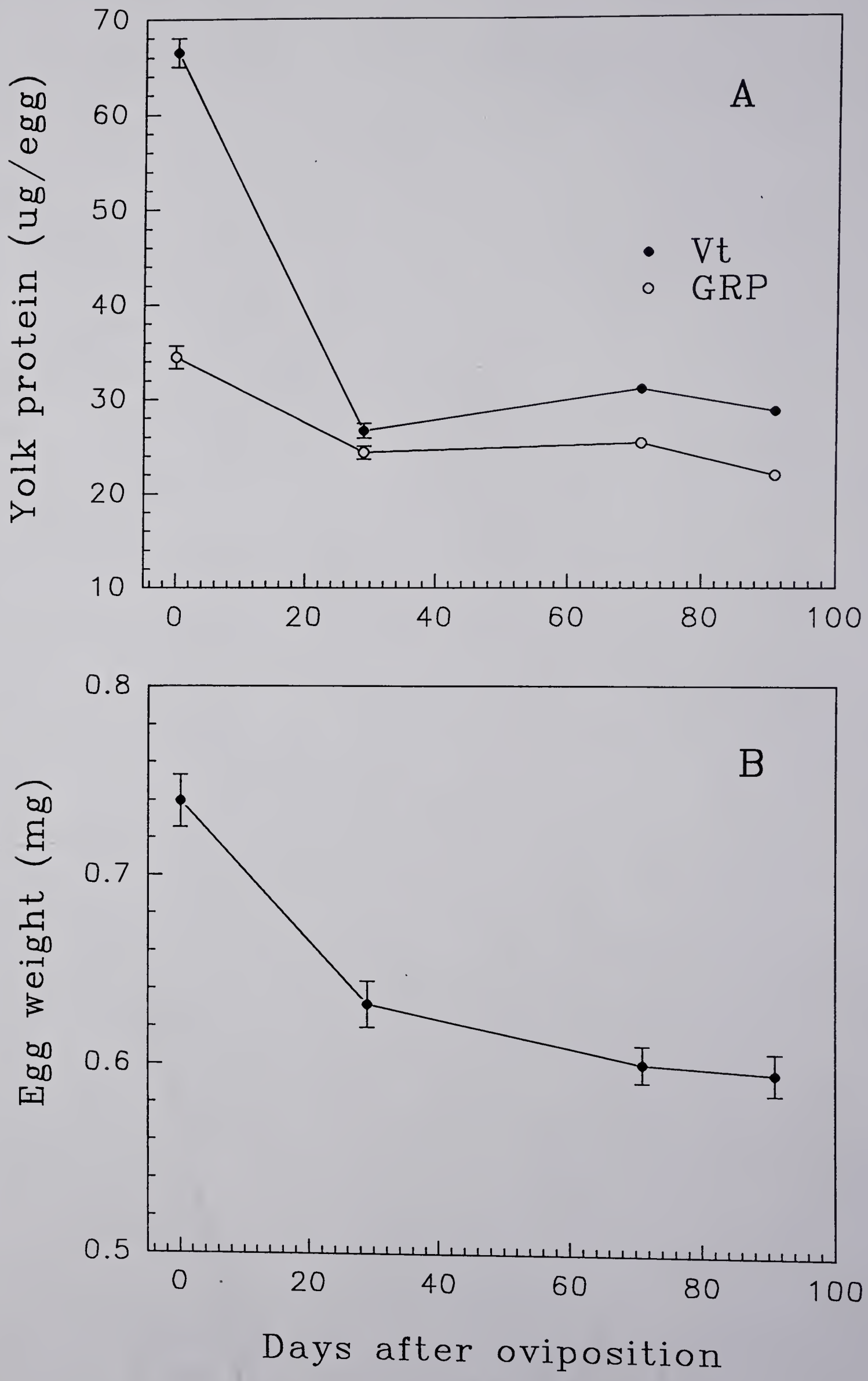

A
Vt
GRP
Yolk protein (ug/egg)
70
60
50
40
30
20
10
0
20
40
60
80
100
B
Egg weight (mg)
0.8
0.7
0.6
0.5
0
20
40
60
80
100
Days after oviposition

Figure 3.2. Changes in average egg weight in the 91 days following oviposition in eggs held under the control regime of 24°C for 28 days, 20°C for 42 days and 12°C for 21 days or at a regime 3°C higher at every step.

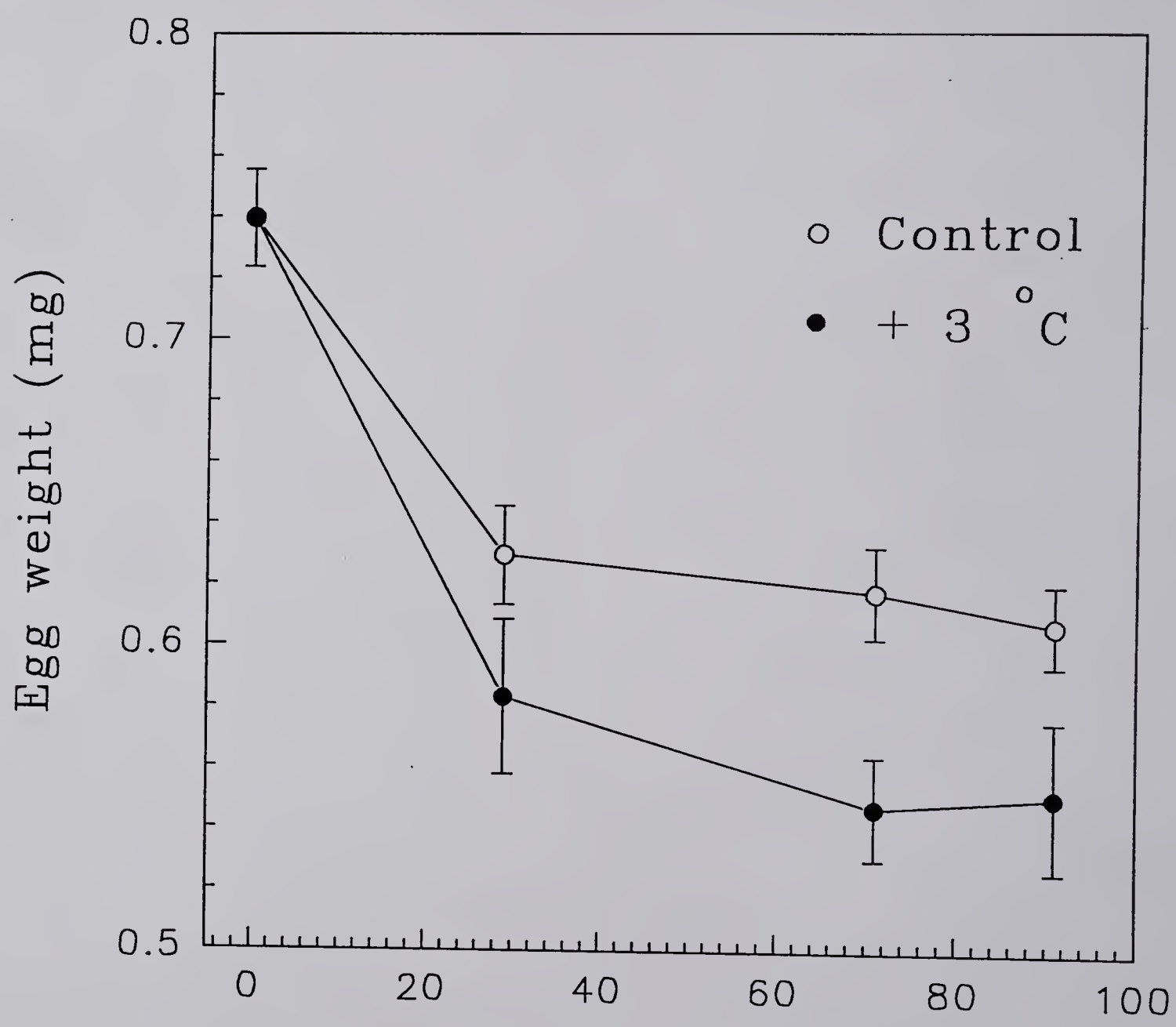

0.8
0.7
0.6
0.5
Egg weight (mg)
Control
+ 3 °C
0
20
40
60
80
100
Days after oviposition

Figure 3.3. Decrease in egg weight in the months following oviposition in eggs in the following four treatments: control temperature regime and control length of test (24°C for 28 days, 20°C for 42 days and 12°C for 21 days), 3°C higher and control length of test (27, 24 and 15°C, changed on above schedule), control temperature regime and extended length of test (24°C for 35 days, 20°C for 49 days and 12°C for 21 days), and 3°C higher and extended length of test.

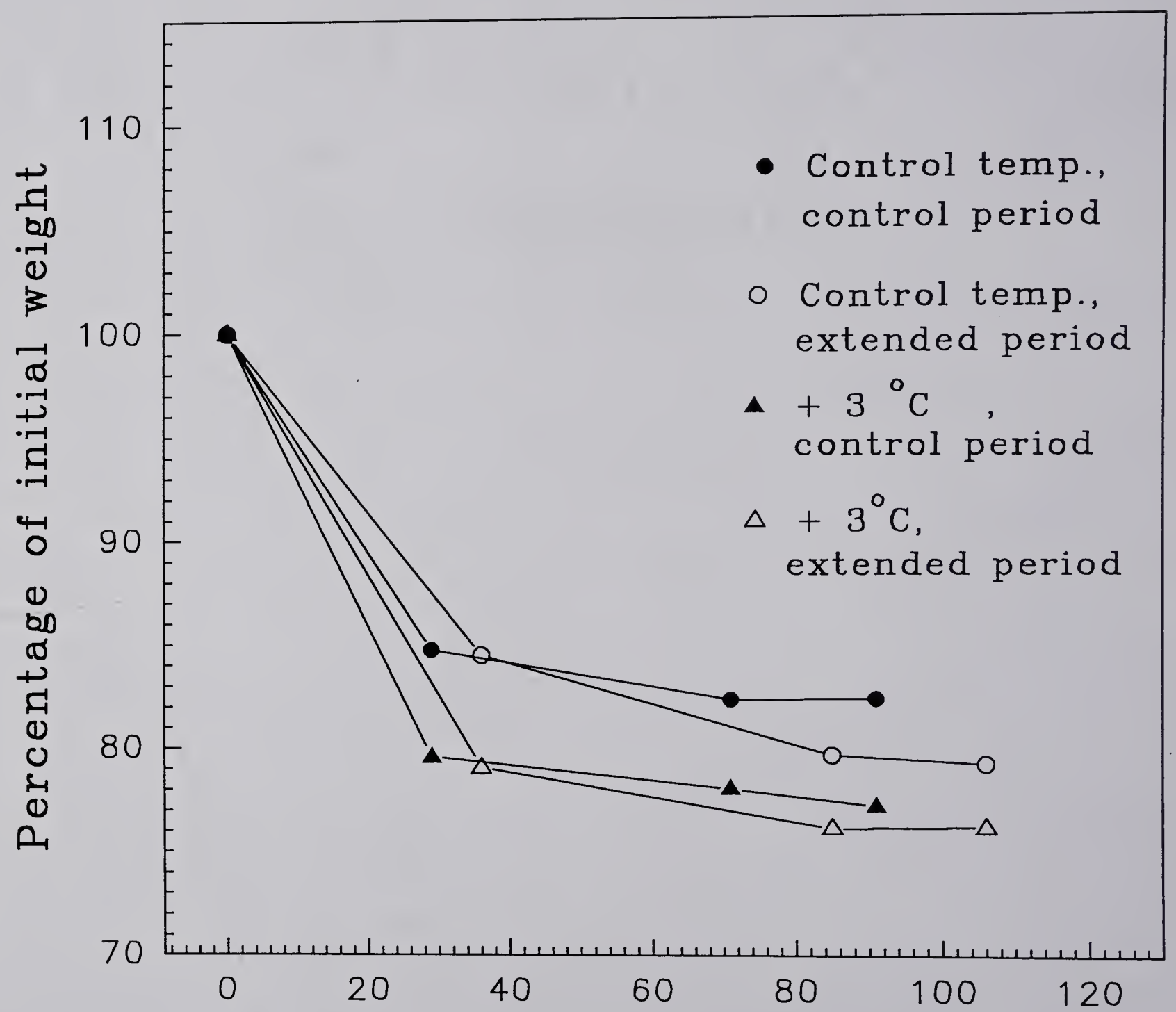

Percentage of initial weight
110
100
90
80
70
0
20
40
60
80
100
120
Days after oviposition
● Control temp., control period
○ Control temp., extended period
▲ + 3 °C , control period
△ + 3°C, extended period

Figure 3.4. Association of pre-hatch levels of Vt with period between placement at 20°C and the eclosion of the first egg(s). Symbols indicate the temperature egg mass sections were held at for six weeks prior to testing. Regressions are of all data depicted in a plot.

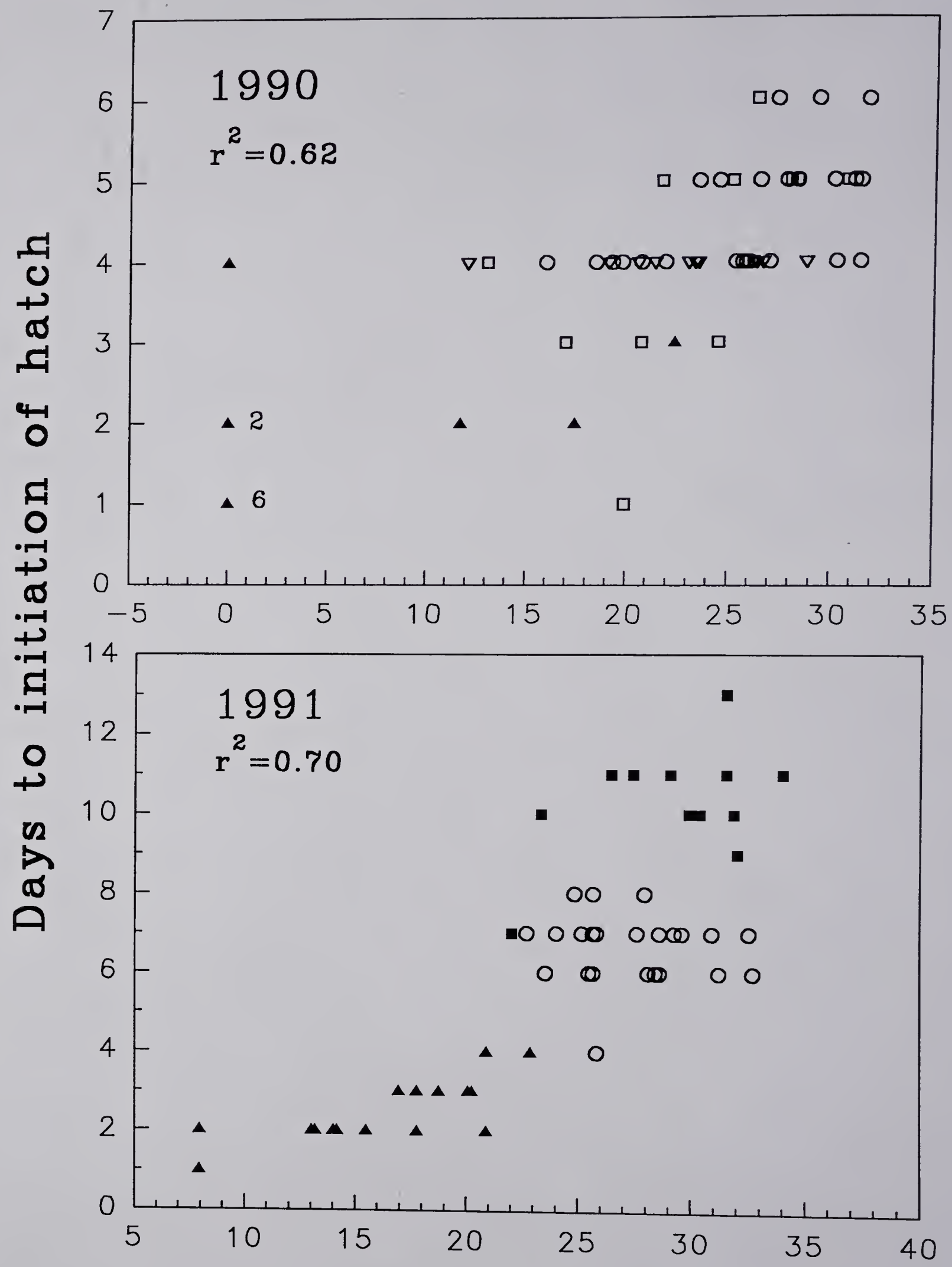
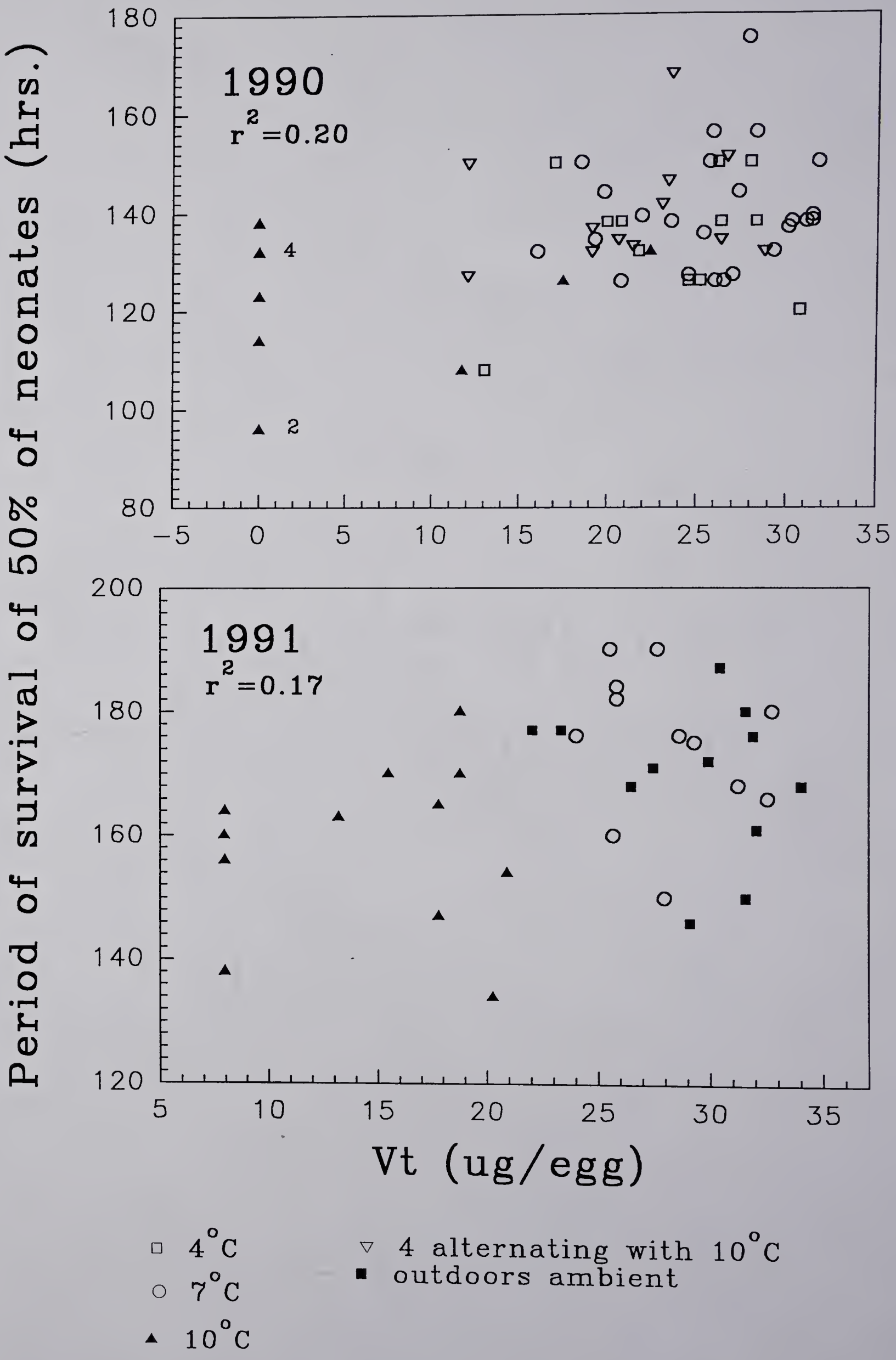

1990
r²=0.62
1991
r²=0.70
Days to initiation of hatch
Vt (ug/egg)
4°C
7°C
10°C
4 alternating with 10°C
outdoors ambient

Figure 3.5. Association of pre-hatch levels of Vt with longevity of 50% of a sample of 60 unfed neonates. Symbols indicate the temperature egg mass sections were held at for six weeks prior to testing. Regressions are of all data depicted in a plot.


1990
r²=0.20
1991
r²=0.17
Period of survival of 50% of neonates (hrs.)
Vt (ug/egg)
4°C
7°C
10°C
4 alternating with 10°C
outdoors ambient

CHAPTER 4

DISTRIBUTION OF YOLK PROTEINS WITHIN THE EGG MASS IN RELATION TO LARVAL ECLOSION, SURVIVAL AND DISPERSAL IN GYPSY MOTH, *LYMANTRIA DISPAR* L.

Abstract

In gypsy moth egg masses from natural populations, levels of the yolk storage protein vitellin (Vt) were progressively lower from first-, center and last-laid thirds of egg masses following oviposition as well as prior to eclosion. Levels of glycine-rich protein (GRP), a recently described yolk protein, were initially similar among egg mass thirds. By eclosion, however, GRP was distributed similarly to Vt. Egg weights were equivalent in the first-laid and center sections, but lower in the last-laid third. Nutritional stress of the maternal generation associated with host defoliation had no effect on levels or distribution of Vt, GRP or weight of eggs. Timing of eclosion and tendency of neonates to disperse were similar in all egg mass sections and influenced by levels of yolk proteins or egg weight. Longevity of

neonates, and thus the length of the window for neonate dispersal, was greater in the first-laid section. The length of neonate survival was positively associated with levels of Vt and GRP in the first and center thirds, but not the last. Defoliation level experienced by the maternal generation had no influence on timing of eclosion, longevity of neonates, or tendency to disperse.

Introduction

In North American gypsy moth, *Lymantria dispar* (L.), adult females cannot fly; dispersal is primarily by first instar larvae. Within days of hatching, larvae climb to the highest point available, spin out on silk, and are borne aloft on breezes in a behavior referred to as ballooning. Although most larvae land within 150 meters of their release point (Mason and McManus 1981, McManus and Mason 1983), a small percentage may be carried many kilometers (Taylor and Reling 1986). Ballooning is generally considered to play a role in range expansion (Elkinton and Liebhold 1990, Cameron et al. 1979, Schwalbe 1981) but its contribution to the spread of outbreaks is a matter of controversy (Liebhold and McManus 1991, Valentine and Houston 1979, Wallner 1986, Leonard 1971). For individual larvae, ballooning represents a possible means of escape from unfavorable hosts or conditions

(Leonard 1970, Lance and Barbosa 1981, Hunter and Lechowicz 1992).

Because neonates disperse prior to the initiation of feeding (Leonard 1971), energetic requirements for dispersal must be met from resources carried over from the egg. I focused on two yolk storage proteins vitellin (Vt), the predominant protein in eggs (Dompenciel 1992), and glycine-rich protein (GRP), a recently described storage protein of gypsy moth eggs (Dompenceil 1992). Both of these proteins are detectable until eclosion, and thus are likely to be available as a resource to neonates.

Eggs decrease in size along the order of oviposition (Leonard 1970), suggesting variation in provisioning of resources. Capinera et al. (1977) found large eggs contain more yolk protein than do small eggs; it is unclear, however, whether they found this variation within egg masses. Rossiter (1991b) suggests that variation in resource provisioning of eggs results in variation among offspring life-history traits, and that this variation could increase the likelihood that a proportion of offspring would survive in an unpredictable environment. Here, I examine variation within egg masses in distribution of egg weight and yolk proteins Vt and GRP, and whether this variation is associated with the life-history traits of timing of eclosion, length of neonate survival and their tendency to disperse. I also examine

whether a maternal effect (the level of defoliation experienced during the parents feeding stage) influenced within mass variation.

Methods

I obtained eggs from females collected as prepupae from field populations in July, 1990 and 1991. I collected additional egg masses from the 1990 and 1991 cohorts in late March after they had overwintered in the field. To reduce variation associated with host species (Capinera and Barbosa 1977), I collected egg masses and female prepupae from boles of oaks in stands dominated by red oaks mixed with lesser amounts of white or chestnut oaks (*Quercus rubra*, *Q. alba*, and *Q. prinus*, respectively). I visually estimated level of defoliation at collection sites at the time of pupation. In 1990 and 1991, six sites were 10-20% and six sites 90-100% defoliated. Five new sites were selected in 1991 and one 1990 site retained. Data are from both defoliated and undefoliated sites unless otherwise stated.

Female prepupae were placed in individual petri dishes at 24^{o}C and a 16:8 LD photophase. Upon eclosion, I moved females to separate paper bags and provided a laboratory reared male (NJ strain). On completion of oviposition, I cut egg masses from bags and numbered them,

marked the last section oviposited, and labeled each mass with a code indicating the site from which the maternal parent was collected.

To determine the initial distribution of Vt, GRP and egg weight within the egg mass, I sampled 72 egg masses from the 1991 cohort the day they were completed by females. These masses were obtained from 12 prepupae each from three sites with light (15%) and three with heavy (90-100%) defoliation, which allowed us to concurrently address whether defoliation experienced by the maternal generation influenced distribution of Vt, GRP and egg weight.

Egg samples for analysis consisted of five eggs taken from the centers of the first-, center and last-oviposited thirds of each egg mass. I dehaired and weighed eggs, then used quantitative (rocket) immunoelectrophoresis (QIEP) (Laurell 1966) as modified by Kunkel (1988) to determine the levels of Vt and GRP in the sample. A description of the production of antisera, preparation of samples, and procedure for electrophoresis is found in Chapter 2.

To determine if distribution of Vt, GRP and egg weight just prior to eclosion was similar to that at completion of oviposition, I repeated the procedure described above using eggs collected from the field in late March. Cohort 1990 was represented by 20 egg masses,

five each from four sites. Two of the sites were heavily (90-95%) and two lightly (15-20%) defoliated. The collection from the 1990 cohort consisted of 72 egg masses, 12 from each of the six sites mentioned above. I held these masses at 7°C, 8:16 LD photophase until testing in April.

I selected 18 egg masses from the 1990 cohort for examination of variation within the egg mass in timing of eclosion, survival and tendency of neonates to disperse. Half of the masses from the 1990 cohort were from three sites with heavy (90-95%) and half from three sites with light (15-20%) defoliation. I obtained these masses from females collected as prepupae as described above and then held them at 24°C for 30 days, 20°C for 40 days, and 12°C for 20 days to approximate natural conditions during late summer and fall. Ninety-one days after oviposition, I placed the egg masses at 7°C and 8:16 LD photophase until April. In April, I divided mass into thirds along the order of oviposition. I took egg samples for protein analysis and weighing as described above. I then placed each third individually in 100 X 15 mm petri dishes with moist dental wicks, at 20°C and 16:8 LD photoperiod to stimulate eclosion. I collected neonates daily at 0900 hrs and recorded the number that eclosed for each section. From each third of an egg mass, I took 120 neonates and

placed them in groups of 10 in 100 X 15 mm petri dishes with a moist dental wick.

I tested 60 neonates from each egg mass section for the length of time of survival without food. Larvae were checked daily at 0900 and 2100 hrs, the number of survivors recorded, and the dead discarded. When a larva was found feeding on another, I removed both and deleted them from the data set. Tests continued until all larvae died. I plotted the number of survivors at each observation against time, and extrapolated the time at which 50% of the sample survived from the curve.

I tested the remaining 60 neonates from each egg mass section for their tendency to disperse in a wind tunnel. Neonates were kept at 20^{o}C, 16:8 LD photoperiod for 48 hours. At 0900 hrs, I moved larvae to the room containing the wind tunnel to acclimate for two hours. Testing began at 1100 and continued until 1500 hrs, the peak period for ballooning in the field (Leonard 1971). A complete description of the wind tunnel and test protocols is found in Chapter 2. In brief, the wind tunnel included a variable speed squirrel-cage fan, a baffle to produce a laminar air flow, and two platforms located side by side in the center of the wind stream. I placed 10 larvae on each platform and allowed them to settle for two minutes. I then exposed them for six minutes in two minute sequences to wind velocities as follows: 1.0 m/sec, 2.1

m/sec, and alternating 1.0 and 2.1 m/sec in 15 second intervals. The tendency of larvae to disperse was represented as the percentage that ballooned from the platform.

Statistics

I used a multivariate ANOVA to determine the effects of order of oviposition (egg mass section), defoliation in the maternal generation, and their interaction on the following: levels of Vt and GRP, egg weight, time to start of hatch and period for 90% completion of hatch, length of survival of neonates, and tendency of larvae to disperse in a wind tunnel. The independent factors of the model were defoliation level, egg mass section, egg mass and the interaction of defoliation level and egg mass section. Error terms for the model were the interaction of defoliation level and site of collection and the residual error. The models for timing of eclosion, neonate longevity and dispersal also included the covariates of Vt, GRP, and egg weight. I tested whether changes in Vt and GRP were due to changes in egg weight using a test for additional information following a multivariate factorial analysis of dispersion (Rao 1965). I used least squares regression to analyze the association of hatch parameters, neonate survival, and tendency to disperse with pre-eclosion protein levels and egg weight.

Results

Egg Weight and Protein Levels

In newly oviposited egg masses, levels of Vt were progressively lower from first- to last-laid sections (Table 4.1). I observed a similar pattern of distribution of Vt in eggs sampled prior to hatch in both the 1990 and 1991 cohorts (Table 4.2). GRP was distributed equally in all three sections of egg masses sampled following oviposition (Table 4.1). By April, however, level of GRP in the last laid section was lower than in the first laid section (Table 4.2, $P \leq 0.01$ in both cohorts). Defoliation level experienced by the maternal generation had no effect on amounts or distribution of Vt, GRP, or egg weight at the completion of oviposition or prior to hatching (Tables 4.1 and 4.2).

The pattern of change in egg weight along the egg mass in samples taken prior to eclosion differed from those of the two proteins. Egg weights were similar in the first- and center, but significantly lower in the last-laid section ($P<0.01$ for both Fall samples). Decrease in levels of Vt and GRP along the order of oviposition in prehatch samples was not due to a decrease in egg weight ($P<0.01$ in both cohorts).

Parameters of Eclosion

Eggs from the three sections of egg masses did not differ in the length of time to initiation of eclosion in April after being placed at 20°C (Table 4.3). The period from first to 90% of total hatch was also similar between sections. Defoliation experienced by the maternal parent did not influence the timing or duration of hatch. Neither the time to initiation nor the duration of the period of hatch was associated with levels of Vt (P=0.24, 0.18 respectively), GRP (P=0.57, 0.48 respectively) or egg weight (P=0.94, 0.95 respectively).

Neonate Survival

Longevity of neonates was significantly higher in the first-laid than in the center and last-laid sections of an egg mass (Table 4.3). Defoliation level had no influence on neonate survival. Levels of Vt and GRP were associated with the length of neonate survival in the first- and center, but not in the last-laid section (Fig 4.1, $P<0.01$ for Vt and GRP in both sections). Vt explained more of the variation in longevity among neonates from sites that had been lightly ($r^2=0.63$) than heavily defoliated ($r^2=0.24$) (data for regressions from first-laid and center sections of the egg mass). Longevity was not influenced by egg weight within egg mass sections (P=0.14, 0.68, and

0.12 in first-, center and last-laid sections, respectively).

Dispersal Behavior of Neonates

The tendency of neonates to balloon in a wind tunnel did not differ between egg mass sections (Table 4.3). Level of defoliation experienced by the maternal generation had no effect on the percentage of neonates that ballooned (mean+/-SE: defoliated sites 19.3+/-2.1, undefoliated sites 19.5+/-1.7). Furthermore, the proportion of neonates ballooning was not influenced by egg weight, level of Vt or GRP (P=0.80, 0.39, and 0.09, respectively).

Discussion

Levels of Vt declined from first- to last-laid sections in newly laid egg masses from field populations of gypsy moth (Table 4.1), a pattern of distribution similar to that in eggs dissected from ovarioles of the New Jersey laboratory strain of gypsy moth (Dompenciel 1992). The pattern of distribution of Vt along the egg mass persisted from oviposition to shortly before eclosion (Table 4.2), though Vt levels were lower in the pre-hatch samples. The maintenence of this pattern from oviposition

to eclosion implies a similar rate of utilization of Vt in all eggs of the mass.

Levels of GRP in eggs following completion of oviposition are similar in all sections of egg masses (Table 4.1). This result is similar to Dompenciel's (1992) finding of no significant change in GRP along the ovariole, though there is a slight increase in the distal portion, corresponding to the last laid section of an egg mass. Mean levels of GRP in the last-laid sections by April were approximately 15% lower than those in the first-laid sections (Table 4.2), suggesting a higher rate of utilization in the last laid eggs.

Freshly laid eggs decreased in weight from first- and center, to last-laid sections (Table 4.1), similar to the pattern of egg size observed by Leonard (1970). In eggs dissected from ovarioles, Dompenciel (1992) found no obvious change in egg weight along the oviduct, but did not weigh eggs in the terminal 1/4th of the oviduct. Surprisingly, egg weights in the pre-hatch samples were equivalent in the first and center thirds of the egg mass, but decreased in the last third. I am confident that this pattern is real; my sample was large (92 egg masses) and the pattern is consistent in both years.

The abrupt decline in egg weight in the last-laid third of egg masses in the pre-hatch sample is likely due mostly to components other than proteins, since proteins

make up only about 10% of the weight of gypsy moth eggs (Dompenciel 1992). Other components which might contribute to the pattern of egg weight include lipids, thickness of chorion, and water content.

Within a mass, change in egg weight did not account for the gradual decline in Vt levels along the order of oviposition. This is not surprising as the patterns of change in egg weight and Vt level are not similar (Table 4.2). That a change in egg weight does not result in a proportional change in Vt level within egg masses indicates that variation in Vt levels within a mass may be greater than would be predicted on the basis of egg weight.

While females from sites that had been heavily defoliated were nutritionally stressed as evidenced by their reduced pupal weights and fecundity (Chapter 2), this experience had no effect on levels or distribution of Vt or GRP. I showed in Chapter 2 that females compensate to maintain an average level of these proteins among their eggs. My results here indicate that while females may differentially provision Vt among their eggs, they maintain the proportion of eggs with high, intermediate and low levels of Vt regardless of the number of eggs they lay. The mechanism by which stressed females maintain both amounts and variation in provisioning of yolk proteins merits further study.

Time to eclosion after eggs were placed at 20°C did not differ among first-, center and last-laid thirds of egg masses. Levels of Vt, GRP, or egg weight had no influence on the timing of eclosion. I found the lack of influence surprising as it appeared to be in conflict with previous observations: Rossiter (1991b) found gypsy moth eggs in her heaviest size classes eclosed earlier and I observed in Chapter 3 that eggs depleted of Vt and GRP eclosed much sooner. It should be noted, however, that these earlier studies only found an effect on timing of eclosion among eggs with extremes of weight or protein level. That I found no influence on timing of eclosion may reflect the more moderate levels of yolk proteins and weight of eggs in my samples.

Dispersal occurs before neonates feed (Leonard 1971); thus the longevity of unfed neonates approximates the period during which dispersal can occur. Longevity of unfed neonates was greater in larvae from the first-laid section of the egg mass in the 1990 cohort (Table 4.3). I showed in Chapter 2 that longevity of neonates correlated with average levels of Vt and GRP for eggs in a mass. Since Vt levels decrease from first- to last-laid thirds of the mass, I expected a similar pattern of decline in longevity of neonates.

Levels of Vt and GRP had a highly significant association with neonate longevity in the first-laid and

center sections of the egg mass: protein levels accounted for 37 to 50% of the variation in longevity of neonates (Fig 4.1). I was surprised to find, however, that the influence of Vt and GRP on longevity disappears in the last laid section of the egg mass. Factors other than Vt and GRP play a role in determining longevity as indicated by the large amount of unexplained variation in longevity. The lack of correlation between Vt and GRP and length of survival in neonates of the last-laid section, however, suggests the influence of other factors on longevity may be greater in that section. It is not clear why the influence of Vt and GRP changes in the last-laid section; the reason for the change, however, might be clarified when other factors affecting longevity are identified.

The tendency to balloon in a wind tunnel was similar in larvae from each third of the egg mass. The lack of association of dispersal behavior with ovipositional order in gypsy moth is similar to that of *Malacosoma californicum pluviale* (Dyar) (Myers 1978). Pre-hatch egg weight, Vt, and GRP levels are not associated with tendency of larvae to disperse in this or my previous study, although the low percentage of larvae ballooning and relatively small amount of variation may make it difficult to detect such associations.

Differential provisioning of eggs has been suggested as a method of increasing variation in offspring

phenotype, particularly in traits expressed at or soon after hatching (Wellington 1965, Leonard 1970, Myers 1978, Capinera 1979, Kaplan and Cooper 1984, Rossiter 1991). My data show gypsy moth yolk proteins Vt and GRP are differentially provisioned among eggs, and that protein levels affect longevity of neonates, likely extending the window for dispersal. I found, however, no evidence for differential provisioning influencing the timing of eclosion or tendency of neonates to balloon.

Table 4.1. Mean (SE) egg weight, Vt and GRP for first-, center and last-laid sections of egg masses on completion of oviposition. Seventy-two egg masses were obtained from females collected as prepupae in July 1991: 12 each from three sites with light (15%) and three with heavy (90-100%) defoliation. P values are from a multiple factor ANOVA: variation between sites within a defoliation class was used as the error term to evaluate defoliation, and residual error to evaluate the other factors. Means on the same row followed by different letters are significantly different at the 5% level using Sheffe's comparison of means.

	First Laid	Center	Last Laid	Section	Defol.	Interaction
Vt (ug/egg)	103.3 (1.2)a	87.9 (1.3)b	74.6 (1.2)c	P<0.01	P=0.65	P=0.46
GRP (ug/egg)	32.8 (0.6)	32.4 (0.6)	32.1 (0.7)	P=0.42	P=0.85	P=0.81
Wt (mg/egg)	0.78 (0.01)a	0.76 (0.01)a	0.71 (0.01)b	P<0.01	P=0.09	P=0.21

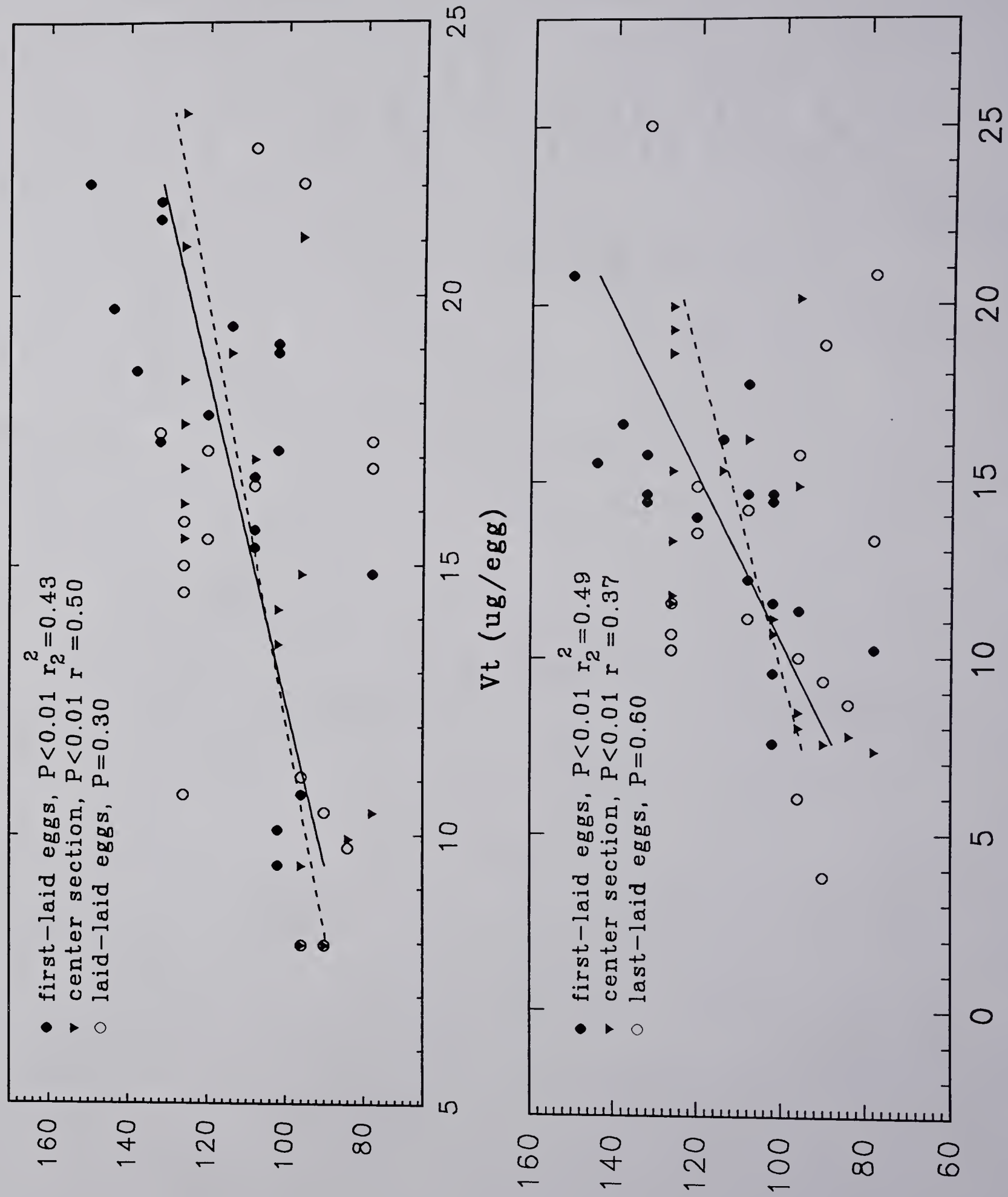
Table 4.2. Mean (SE) egg weight, Vt and GRP for first-, center and last-laid sections of egg masses prior to eclosion. Twenty egg masses from four sites contributed to the means in cohort 1990 and 72 egg masses from six sites in the 1991 cohort. In both cohorts, sites are equally represented and half were lightly (15-20%) and half heavily (90-100%) defoliated the previous summer. P values are from a multiple factor ANOVA: variation between sites within a defoliation class was used as the error term to evaluate defoliation, and residual error to evaluate the other factors. Means on the same row followed by different letters are significantly different at the 5% level using Sheffe's comparison of means.

	First Laid	Center	Last Laid	Section	Defol.	Interaction
1990 Cohort						
Vt (ug/egg)	19.6 (1.1)a	17.6 (0.8)a	14.4 (0.8)b	P<0.01	P=0.26	P=0.63
GRP (ug/egg)	15.0 (0.8)a	13.9 (0.7)ab	12.4 (0.8)b	P=0.02	P=0.29	P=0.84
Wt (mg/egg)	0.69 (0.02)a	0.69 (0.02)a	0.58 (0.03)b	P<0.01	P=0.89	P=0.88
1991 Cohort						
Vt (ug/egg)	27.1 (0.7)a	24.8 (0.6)b	20.6 (0.7)c	P<0.01	P=0.12	P=0.88
GRP (ug/egg)	18.0 (0.4)a	17.0 (0.4)b	15.7 (0.4)c	P<0.01	P=0.30	P=0.68
Wt (mg/egg)	0.67 (0.01)a	0.67 (0.01)a	0.60 (0.02)b	P<0.01	P=0.07	P=0.99

Table 4.3. Mean (SE) days to initiation of hatch, duration of hatch, percentage of larvae ballooning and longevity of unfed neonates from the first-, center and last-laid sections of egg masses. Longevity is expressed as the number of hours 50% of a sample of 60 larvae survived. Eighteen egg masses contributed to means, three from each of six sites. Half of the sites were lightly (15%) and half heavily (90-100%) defoliated. Probability values for the factors of egg mass section, defoliation level and their interaction were taken from a multiple ANOVA which also included egg mass and the covariates of Vt, GRP and egg weight. Means on the same row followed by the same letter are similar at the 5% level using Sheffe's comparison of means.

	First Laid	Center	Last Laid	Sect.	Defol.	Sect.*Defol.
Days to initiation of hatch	3.1 (0.1)	3.1 (0.1)	3.2 (0.1)	P=0.25	P=0.49	P=0.99
Duration of hatch (days)	2.3 (0.1)	2.2 (0.2)	2.1 (0.1)	P=0.23	P=0.73	P=0.94
Percentage Ballooning	17.7 (2.4)	21.5 (1.9)	16.5 (2.4)	P=0.30	P=0.99	P=0.14
Longevity (hrs)	114.0 (4.1)a	107.1 (3.6)b	104.1 (4.0)b	P<0.01	P=0.32	P=0.21

Figure 4.1. Plots of longevity of unfed neonates against levels of Vt and GRP within first-, center and last-laid thirds of the egg mass. Regressions for data from the first-laid section are shown as solid lines, dashed lines are regressions of data from the center section.


first-laid eggs, P<0.01 r_2^2=0.43
center section, P<0.01 r =0.50
last-laid eggs, P=0.30
first-laid eggs, P<0.01 r_2^2=0.49
center section, P<0.01 r =0.37
last-laid eggs, P=0.60
Vt (ug/egg)
GRP (ug/egg)
Longevity of unfed neonates (hours)

CHAPTER 5

CONCLUDING REMARKS

Compensation of Vt and GRP

One of the most interesting results of my study is that nutritionally stressed females compensate to maintain similar levels and distribution of Vt and GRP as unstressed females. This compensation is important because these proteins are associated with longevity of neonates as well as being used by the pharate larva prior to eclosion and in the formation of the embryo. Females do not compensate to maintain all egg resources, however, as reflected in lower average weight of eggs from nutritionally stressed females.

Role of Vt and GRP in Ballooning and Longevity

I was surprised to find that Vt and GRP do not influence the tendency of neonates to balloon. Perhaps these proteins do not provide the resources for ballooning, or dispersal is not very energetically demanding. Neonate longevity, in contrast, is positively associated with levels of Vt and GRP. It seems unlikely that Vt and GRP are the only egg resources used; these proteins account for at most about 50% of the variation in longevity and longevity of neonates from the last-laid section of egg mass is not associated with levels of Vt or GRP, as in other sections of the egg mass. The absence of the influence of Vt and GRP on longevity in the last-laid third of the egg mass suggests

that research on the relationship between neonate survival and other resources might best be done using these eggs.

Effect of Maternal Nutritional Experience

Interestingly, the tendency to balloon is less in offspring of stressed females. The relationship between maternal stress and larval dispersal might be clarified by partially starving female larvae at different levels and testing their offspring in a wind tunnel.

The decreased tendency of ballooning among neonates of stressed females suggests the influence of another egg resource, one that females do not compensate to maintain as they do for Vt and GRP. This resource is likely to be equally distributed among eggs since the percentages of neonates ballooning from the three egg mass sections were similar.

Nutritional stress of the maternal generation has no affect on neonate longevity. This is not surprising since stressed females compensate to maintain levels of Vt and GRP, both of which are associated with longevity of neonates.

Patterns of Provisioning among Eggs of a Mass

Vt, GRP, and egg weight all change along the order of oviposition, affirming speculation that females in field populations produce offspring with varied levels of egg resources. The effect this has on dispersal, however,

appears small: I noted no difference in the tendency of larvae to disperse from the three sections of the egg mass. Neonates from the first-laid section of the mass survive longest, though the differences between average longevities of the first- vs. the middle- and last-laid sections were 7 and 10 hours, respectively.

Effect of Temperature

I found that temperature has no effect on utilization of the yolk proteins Vt and GRP during embryogenesis and early diapause. Higher temperatures during the post-diapause period in early spring, in contrast, result in reduced levels of Vt and GRP in eggs prior to eclosion. Longevity of neonates from eggs held at higher temperatures in early spring is also reduced. In the fall, preventing excessive consumption of Vt and GRP needed for survival of neonates would seem to be advantageous. In the spring, utilization of these proteins appear to be involved in preparations for eclosion.

Further Research

In addition to the potential research topics mentioned above, I make the following suggestions.

For those who wish to study protein content of eggs or hemolymph as it relates to insect population ecology, I suggest that the ELISA test be used. While ELISA requires purification of antigen prior to injection into the animal,

it uses much smaller amounts of the resulting antisera and facilitates the processing of the large number of samples necessary for population studies.

It would be interesting to determine whether temporal differences in the tendency to balloon exist between neonates from stressed and unstressed females. This question could be addressed by collecting a cohort of neonates from an egg mass and daily selecting a subsample for testing in the wind tunnel. The percentage of neonates ballooning on each day following exclosion from egg masses of stressed and unstressed populations could then be compared.

The question of whether neonates differ in their frequency of ballooning is still unresolved. It would be particularly interesting to determine if maternal stress influences the number of times their offspring will balloon. Unfortunately, I cannot suggest a methodology since neonates that are re-tested are too active to remain on the platforms in the windtunnel.

I attempted to determine the sex ratio among larvae that ballooned and those that did not. I found the sexes equally represented in both groups. Many larvae from field-collected masses died from disease, however, and it is impossible to determine whether differential mortality influenced the sex ratio.

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