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Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L.

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Abstract North American gypsy moths 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. We 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 and vitellogenin in the maternal parent during the prepupal stage had no influence on levels of yolk proteins, larval longevity, or tendency to balloon.

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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 (Leonard 1971a; Cameron et al. 1979; Elkinton and Liebhold 1990; Leibhold and McManus 1991).

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 m of their take-off 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; D.E. Leonard, personal communication), 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 (Capinera and Barbosa 1976; Barbosa et al. 1981; Lance and Barbosa 1981).

Rossiter (1991, 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 one third of the initial amount of GRP remains in eggs at the time of hatch.

We 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 we focused on were arylphorin (Ap), the predominent protein in the hemolymph (Karpells et al. 1990) and Vg, the precurser to Vt (Dompenceil 1992). We investigated whether levels of these hemolymph proteins could be used to predict levels of Vt and GRP in eggs just prior to hatch. We 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, we considered whether levels of Vt and GRP or weight of eggs were related to longevity of first instars or their tendency to balloon.

Materials and methods

Preparation of antisera

We prepared a polyclonal antiserum against gypsy moth egg proteins including Vt and GRP using the method of Kunkel (1988). We dissected chorionated eggs from unmated females and homogenized 1 g of eggs in 8 ml of PBS (0.15 M NaCl, 0.10 M Na_2HPO_4 pH 7.2) and PMSF (phenylmethylsulfonyl fluoride). We centrifuged the mixture at 12000 *g* for 10 min at 4°C and decanted the extract of yolk proteins. We 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, we gave the goat a booster injection of the antigen with incomplete Freund's adjuvant. A week after the last injection, we prepared serum from the goat and measured the antibody titer. We 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% $NaNO_3$. We separated the antiyolk serum into 5 ml aliquots and stored them at –20°C until use. We titered Ap using polyclonal antiserum characterized previously (Karpells et al. 1990).

Protein determination

We 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 × 11 cm glass slide with 10 ml of a 1% agarose gel (Bio-Rad Standard low-Mr) containing a known percentage of the appropriate antiserum. We held slides prior to sample loading for no longer than 3 h in a chamber at high humidity to minimize loss in volume from evaporation that could affect the height of precipitation peaks. We punched wells that held 3.3 µl of fluid sample in the gels immediately before loading them. We 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.

We combined samples of five eggs each with 500 µl 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 1000 microhomogenizer. We held egg homogenates on ice to prevent degradation of proteins, and loaded samples of the fluid portion into the gels within 15 min.

We took hemolymph samples immediately after collection of female prepupae from the field. Holding prepupae in a supine position, we pricked one proleg with a fine pin and collected 2 µl of hemolymph from the wound using a microcapillary tube. Prepupae recovered from the procedure with no apparent harm.

We prepared protein extracts of hemolymph immediately after the hemolymph was collected. Samples of hemolymph (2 µl) were expelled into individual 1.5 ml microcentrifuge tubes, and diluted with 198 µl of a 50/50 mix of gycerol and TEC saturated with phenythiourea to prevent melanization. We centrifuged samples at 6000 rpm for 10 min at 4°C to precipitate hemocytes and stored them at –20°C for up to 2 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 con-

sisted of pooled hemolymph from female prepupae diluted to 50% with glycerol to prevent protein denaturation. Aliquots of standard were stored at –20°C and diluted with TEC before use.

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

We identified the specific responses of the antiyolk serum to Vt and GRP using purified samples of these proteins. We measured the area under protein precipitation peaks, or "rockets" using a Bio-Rad Peak Height Area Estimator. We 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

We collected egg masses and prepupae in Massachusetts and Vermont from sites with either high or low levels of defoliation. We assumed that larvae that matured at defoliated sites had experienced higher levels of nutritional stress. Populations we 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 10–20% defoliation of the canopy. Defoliation was visually estimated during the first week of July when larvae were in their last instar.

We 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 1 km. 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 1).

Table 1 Percent defoliation and tree species composition at each collection site. Sites within the Millers Falls township were separated by at least 1 km

Year	Defoliation	oak	Proportion of species	Locality
Low defoliation sites				
1990	20%	50% red, 50% chestnut		Miller's Falls, Mass.
1990	15%	60% red, 40% chestnut		Amherst, Mass.
1990	10%	40% red, 60% white		North Hero Is., Vt.
1991	15%	50% red, 50% white		Belchertown, Mass.
1991	15%	50% red, 50% white		Hadley, Mass.
1991	20%	10% red, 90% white		Granby, Mass.
High defoliation sites				
1990	95%	50% red, 50% white		Gill, Mass.
1990	90%	90% red, 10% white		Miller's Falls, Mass.
1990	99%	50% red, 50% white		Deerfield, Mass.
1991	95%	50% red, 50% white		Gill, Mass.
1991	90%	50% red, 50% chestnut		Miller's Falls, Mass.
1991	100%	100% red		Miller's Falls, Mass.

Collection and handling of prepupae and eggs

After sampling for hemolymph, we placed prepupae in separate petri dishes at 24°C and a 16:8 L:D photoperiod to pupate. We weighed female pupae 3 days after pupation. On eclosion, we placed each female in a paper bag with a laboratory reared male (standard lab strain NJSS) to mate and oviposit. We considered oviposition to be complete when the female moved off the egg mass, usually within 3 days of mating. We 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.

We also collected egg masses from the field in late March from the same sites from which prepupae were collected the previous summer. We 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.

We 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, we 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, we staggered hatching of egg masses over a 3-week period between the 2nd week of April and the 1st week of May. We 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, we kept individual egg masses in petri dishes with a moistened dental wick at 20°C, and 16:8 L:D photoperiod. Eggs were checked daily at 0900 hours and the neonates counted and removed.We 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; A.D. Diss, unpublished data). For this reason, each egg mass was subsampled for Vt and GRP in three places. We took samples of five 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°C to promote hatch. Eggs were stored at –20°C, until testing (within a week). We calculated average levels of Vt and GRP for the mass from the values obtained from the three samples. Eclosion typically occurred within 2–6 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 15 × 100 mm petri dish with a moistened wick at 20°C and 16:8 L:D photoperiod. We checked larvae twice daily, at 0900 and 2100 hours and recorded the number of surviving larvae. Moribund neonates were gently prodded with a fine brush; those that did not respond we 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. We determined the longevity of neonates from each mass by summing the total number surviving at each observation, plotting these values against time, and interpolated from the sigmoid survival curve the time at which 50% of the initial population remained alive.

Test of tendency of neonates to disperse

We tested the tendency of larvae to balloon in response to air movement in a wind tunnel with length × width × height of

120 × 25 × 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 different settings of the reostat was calibrated using a hot-wire anemometer (Yokogawa, type 2141 JIS C 1102, class 1.5).

We exposed larvae on platforms of a 9-cm-diameter card mounted on an 11-cm dowel at a 30° 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°C and 40–60% relative humidity. Lighting was from two ceiling mounted fixtures of two 4-ft, 34-W, cool white, fluorescent bulbs.

We determined the tendency of larvae to balloon from a random sample of 60 neonates from each egg mass selected during the 3 days of peak hatch. After eclosion, we held neonates for 2 days 10 to a 15 × 100 mm petri dish with a moist wick at 20°C and 16:8 L:D photoperiod.

We tested larvae 48 h after they had hatched. At 0900 hours, we removed the wicks and placed larvae in the wind tunnel room to acclimate. We conducted tests between 1100 and 1500 hours, the peak period for ballooning in the field (Leonard 1971a). We placed 10 larvae on each of the two platforms in the wind tunnel to acclimate for 2 min in still air. The test took a total of 6 min. The fan was turned on and wind speed was gradually increased to and maintained at 1.0 m/s for 2 min, then 2.1 m/s for 2 min. For the final 2 min of the test, we alternated the air speed between 1.0 and 2.1 m/s in 15s intervals. We recorded the number of larvae remaining on the platforms, then removed and discarded those in the wind tunnel. We 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 analyses

We 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 non-defoliating populations differed in the variables mentioned above. We 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. We used Pearson correlations to determine relationships between egg weight, Vt and GRP content. Statistical calculations were done using BMDP (BMDP 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). 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).

Table 2 Mean level of vitellin (*Vt*), glycine-rich protein (*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 weight (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 weight (gm)[a]	0.704 (0.02)	1.245 (0.01)	<0.01

[a] Calculated from 177 pupae from defoliated and 121 from undefoliated sites collected in 1991

Although defoliation had a pronounced effect on female pupal weights and fecundity, its effect on the egg characteristics measured was not consistent (Table 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°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°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 2–4 days after being placed at 20°C, and 70% had their first eggs hatch on day 3. The range in delay of hatch was much greater in the 1991 cohort. The start of hatch varied between 3 and 8 days after eggs were placed at 20°C, and 80% of masses had their first eggs hatch within days 4, 5, and 6.

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 2 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 h (SE = 5.04, $n = 55$ egg masses) compared with 152.4 h (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 our 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 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 1 shows the positive relationship between neonate survival without food and the amount of Vt and GRP. Neither egg weight nor the number of eggs in a mass had a significant effect on longevity in the 1991 cohort (Table 3). Results of the stepped regression of the smaller 1990 cohort indicated that neither Vt, GRP, egg weight, nor the number of eggs in the mass made a significant addition to the model (Table 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 h. The amount of variation in longevity, however, was similar between the two cohorts ($P = 0.42$).

Table 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)

Character	Probability level	
	1990 ($n = 19$)	1991 ($n = 70$)
Vt	$P = 0.57$	$P = 0.01$[a]
GRP	$P = 0.54$	$P < 0.01$, $r^2 = 0.12$
Egg weight	$P = 0.97$	$P = 0.31$
Number eggs	$P = 0.99$	$P = 0.65$

[a] Probability value before inclusion of GRP in the regression equation

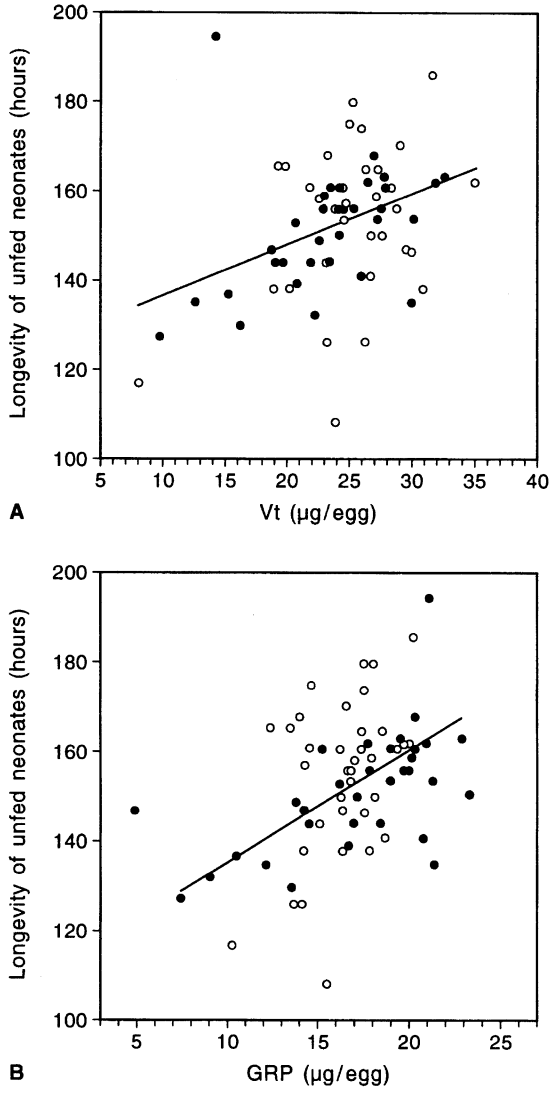

Fig. 1 Relationship between average levels of vitellin (*Vt*; **A**) and glycine-rich protein (GRP; **B**) in eggs of a mass and the longevity of unfed neonates from that mass. **A** $P = 0.04$, $r^2 = 0.06$, $n = 70$. **B** $P = 0.006$, $r^2 = 0.10$, $n = 70$. Data from 1991 cohort, values of these linear regressions differ slightly from those of the stepped regressions described in the text

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 non-defoliating 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 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 the tendency of neonates to balloon in the 1990 cohort (Table 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 ($P = 0.11$).

Table 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)

Character	Probability level	
	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	$P = 0.09$	$P = 0.01$, $r^2 = 0.09$

Discussion

Results of earlier studies suggest that nutritional experience of the maternal parent might affect the likelihood 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 1991, 1992). Our 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). 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.

We were 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 et al. 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 (R.E. Dompenciel, personal communication). Whether nutritional stress or genetic factors affect the timing of production or utilization of these proteins is not known, though we 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). 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). Our data are the first, to our 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). 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 occurs 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 2 or 3 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 more 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. We 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 3, Fig. 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 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 4).

How nutritional stress of the maternal parent is influencing the tendency of offspring to balloon is not clear from our 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 (A.L. Diss, unpublished data). 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%. Our 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 we 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.

In 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 our 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.

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