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MATERNAL EFFECTS IN GYPSY MOTH: ONLY SEX RATIO VARIES WITH POPULATION DENSITY

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Abstract. A number of species of forest caterpillars fluctuate in density with a periodicity of 8–11 yr. One explanation for these ''cycles'' is that changes in food quality or quantity and crowding influence the growth rate and final size of moths at high density. Carryover of these influences to the next generation through maternal effects could modify the dynamics of the population. To determine whether development, survival, pupal size, fecundity, or sex ratio varied among offspring of gypsy moths from high- and low-density populations, we collected eggs from three sites where moths had been at low density and three sites where moth density had been high for several years. We reared caterpillars hatching from these eggs in the laboratory under controlled conditions. Sex ratio was the only characteristic to vary in a consistent way with the density of the maternal population. Egg masses from low-density sites produced significantly more females than those from high-density sites, for which the numbers of males and females were equal or slightly in favor of males. The female bias of the sex ratio of low-density populations increases the potential rate of increase of the population and may arise from local mate competition when populations are sparse. The reduced rate of increase in high-density populations associated with the higher production of males could delay the recovery of populations following decline. Whether the sex-ratio deviation observed in low-density populations persists should be studied in the future.

Key words: forest caterpillars; gypsy moth; local mate competition; Lymantria dispar; *maternal effects; population cycles; population density effects on subsequent generations; population density in gypsy moths; sex ratio.*

INTRODUCTION

Populations of forest caterpillars often fluctuate with a periodicity of 8–11 years. Delayed recovery of the rate of increase for several generations after decline of the populations causes these cyclic dynamics (Myers 1988). A recent model of a maternal-effects hypothesis by Ginzburg and Tanneyhill (1994) includes a ''quality variable'' that provides a lag in recovery and thus generates cyclic dynamics. But various biological mechanisms could lag behind changes in population density and act as a ''quality variable.'' Continued high parasitism for several generations could delay recovery of populations (Berryman 1996), but does not explain reduced fecundity of declining populations (Myers and Kukan 1995, Myers 1996). Reduced food-plant quality could also persist for several years after defoliation and delay the recovery of insect populations by reducing survival, growth, and fecundity. Baltensweiler and Fischlin (1988) interpreted the population dynamics of larch budmoth, *Zeiraphera diniana* Gn, in this way. But not all cyclic increases of forest Lepidoptera cause defoliation (Myers 1993), and therefore impacts on trees can vary. Continued disease contamination could reduce survival and fecundity of insects for several generations (Rothman and Myers 1994). High infection has not always been recorded in outbreak populations of forest Lepidoptera, but monitoring procedures may not have been sufficient to properly quantify viral disease (Kukan and Myers 1995).

Another explanation for delayed recovery of populations is environmentally based maternal effects (Wellington 1957, Rossiter 1991*a*, *b*, 1992, reviews in 1994, 1995). The ''maternal-effects hypothesis'' for herbivore outbreaks states that over a suitable environmental range, the ''quality'' of the individuals in a population will influence its size in the current generation as well in subsequent generations (Rossiter 1994). We distinguish environmental and maternal influences in the following way. Environmental influences act directly on individuals, but the phenotypic variation they cause is not necessarily transmitted to the next generation. Maternal influences, however, modify the behavior or quality of the offspring in subsequent generations. They may be caused by environmental forces acting on the mother, but will be expressed in offspring regardless of the environment, and may be carried over for several generations. Examples of maternal effects are: the increased ability of autumnal moth (*Epirrita autumnata*) caterpillars to feed on foliage with induced

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defensive chemicals if their mothers originated from a high-density population (Haukioja and Neuvonen 1987); the variation in egg size and associated cold tolerance in spruce budworm, *Choristoneura fumiferana* (Harvey 1985); the variation of western tent caterpillar (*Malacosoma californicum pluviale*) activity and survival with the history of the mother in such a way that food-stressed mothers produce less active caterpillars (Wellington 1965); and the variation in survival of offspring with food quality of the parental generation of fall webworm, *Hyphantria cunea* (Morris 1967).

For gypsy moth, *Lymantria dispar*, a large body of evidence shows that high population density precipitates changes in the ballooning behavior of larvae (Leonard 1968, Leonard 1970, Diss et al. 1996), increases developmental rate (Leonard 1968, Campbell 1978, Greenblatt and Barbosa 1980, Lance et al. 1986) and decreases fecundity (Campbell 1967, Leonard 1968, 1970).

Rossiter (1991*a*) distinguishes non-nutritional factors (crowding, temperature) from nutritional (leaf quality) factors that influence the parental generation of moths and then, primarily through the quantity or quality of egg provisions, increase phenotypic variation in the life-history traits of offspring. Offspring phenology, growth rate, and fecundity are examples of traits that can vary. In Rossiter's studies, gypsy moths that fed on trees with greater herbivore damage produced offspring that were heavier as pupae (female pupae were ~25% heavier and males ~10% heavier with an increase in defoliation from 0 to 50%). Increases in condensed tannin levels in the maternal diet were associated with more rapid settlement and initiation of feeding of offspring by ~1.5 d. In Rossiter's study maternal responses to increasing population density and defoliation improved the condition of their offspring and would potentially accelerate population increase. This is the opposite of the results of Wellington (1965), Morris (1967), and Leonard (1970) in which food stress reduced offspring quality.

We predicted that if maternal effects are a robust influence on population dynamics of gypsy moth, caterpillars from high- and low-density populations should vary when reared under constant laboratory conditions. Based on Rossiter's results (1991*a*) we predicted that pupae developing from eggs from high-density populations would be heavier than those from low-density populations when larvae were reared on a standard diet. However, if high population density reduced the allotment of nutrients for egg development (Wellington 1965, Morris 1967, Leonard 1970), we expected the opposite—reduced size, survival and/or slower development of offspring from high-density populations.

In this study we compare the development rate, survival, pupal mass, fecundity, and sex ratio of laboratory-reared gypsy moth caterpillars from egg masses collected from three high-density and three low-density populations in western Massachusetts in the spring of 1994.

Materials and Methods

Study sites

All source populations were within 5–20 km of Amherst, Massachusetts (USA). The three low-density sites are in the Caldwell Forest in the vicinity of the Quabbin Reservoir, Pelham, Massachusetts, in Franklin, Hampshire, and Worchester counties. These sites are 6–20 km apart, beyond the migration distance of flightless females, and are 10–15 km from the high-density sites. The most northern of the sites is Montague, ~20 km north of Amherst along State Route 63. The nearest adjacent site to Montague is Quabbin 20, ~15 km to the southeast. The most southern site is Ludlow, in Ludlow State Park. The closest adjacent site to Ludlow is Quabbin 17, ~12 km to the northeast. The Hoyoke high-density site is on Route 116 ~15 km north of Ludlow, 25 km south of Montague, and 15 km west of Quabbin 4. Although the low-density sites are all indicated as being near the Quabbin Reservoir, they are geographically as close to the high-density sites as they are to each other. Therefore, they can be considered to be independent populations. Woodlands at all of these sites contain a high proportion of northern red oak, *Quercus rubra*, which is a favored host of gypsy moth.

History of populations

Estimates of population density were made at the sites by counting egg masses on trees of all species and on the ground in plots of various sizes. At Quabbin, five 0.01-ha circles were surveyed in four different, 1-ha plots in the three areas (total of 60 circular areas per site). In the summer of 1992 no egg masses were found at any of the Quabbin sites, and in 1993, following the period of moth oviposition, there were 2.8 ± 2.4 egg masses/ha (mean ± 1 SE) for Quabbin 17, 11.3 ± 4.6 for Quabbin 20 and 25.5 ± 10.7 for Quabbin 2. For the Ludlow site egg-mass density estimates are based on searches in 32 plots of 0.02 ha, and in 1993 the density was 1590 ± 303 egg masses/ha. At Holyoke the egg masses were counted in four circles of 0.04 ha and the egg mass densities for two plots were 2612 ± 188 egg masses/ha, and for another two plots 2100 ± 425 egg masses/ha. Egg masses were not counted for the Montague site (but see below and Table 1 for other information on density). Therefore, egg-mass densities in the year of the experiment were approximately 7 to 10 times higher at the high-density sites than at the low-density sites, and Quabbin 17 had the lowest density and Holyoke the highest density. See Elkinton et al. (1996) for further description of populations and techniques.

In addition to counting egg masses to monitor pop-

TABLE 1. Lengths of egg masses (mean ± 1 SE) and estimated number of eggs per mass based on the regression $\log_{10} y = 1.58 \log_{10} x + 0.29$ (Moore and Jones 1987) for egg masses laid in 1992, two summers before the experiment, and in 1993, the summer before the experiment, in the Caldwell Forest near Quabbin Reservoir (Pelham, Massachusetts, USA). Egg masses used in the experiments were laid in 1993 and collected in the spring of 1994. *N* = no. of egg masses; NA = population density was too low to allow collection of eggs. See *Materials and methods: History of populations* for density estimates.

	Egg masses						
	1992			1993			
Study site	Length (cm)	*N*	Est. no. of eggs	Length (cm)	*N*	Est. no. of eggs	Population history
Low density							
Quabbin 17	NA			4.40 ± 0.17	27	770	Low density since 1985, increasing 1993
Quabbin 20	NA			3.89 ± 0.17	28	634	Low density
Quabbin 2	NA			4.50 ± 0.17	34	798	Low density
High density							
Holyoke	3.42 ± 0.14	15	517	1.96 ± 0.06	25	215	Defoliation for 5 yr
Ludlow	3.27 ± 0.16	22	482	2.57 ± 0.09	27	329	Near defoliation in 1992 and defoliated in 1993
Montague	2.71 ± 0.13	25	358	2.65 ± 0.10	25	346	Defoliated at least 1 yr

ulation density, the length of egg masses was also measured. Female moths lay their eggs in a single mass over a 2–3 wk period in late summer, and these remain on the tree or substrate over the winter. The size of the egg mass reflects the fecundity of moths. Population densities are reflected in the size of the egg masses, with high caterpillar densities being associated with smaller egg masses. The lengths of egg masses laid in the summer of 1993 and collected in the spring of 1994, and of those laid in 1992 and collected in 1993 are given in Table 1. At the Quabbin sites the densities were too low in 1992 to permit collection of egg masses, and egg masses laid in the summer of 1993 were relatively large, which reflects the low density. Egg masses laid in 1993 at two of the high-density sites were smaller than those laid in 1992, and egg masses from all three high-density areas were smaller (shorter) than those from the low-density sites ($F = 26.2$, $P < 0.01$, df = 5, 56).

Egg hatching, larval rearing, and egg collection

Eggs were collected from the six sites between 11 and 20 April 1994. At the time of collection the lengths of the egg masses were measured and the tops were marked with a pen to record which were the first eggs laid. The eggs were stored in the refrigerator at 4°C until 26 April when they were soaked for 1 h in a 10% formalin solution, rinsed 1 h in running water, and spread to dry overnight. On 27 April 10 or 12 (Holyoke) egg masses per population were cut in half and each half of each mass was placed in a 2-ounce (50 mL) plastic cup in a controlled-temperature room at 23°C. Eggs began to hatch on 28 April and the last eggs hatched on 10 May. Approximately 13 caterpillars were collected from each of the top and bottom halves of the egg masses, and placed in individual plastic cups of synthetic diet provided by the USDA-APHIS Laboratory (Otis Air National Guard Base, Massachusetts, USA). Containers were stored on racks in a controlled-environment room. The location of the racks was shifted daily to compensate for any variation in environment associated with position in the room. Caterpillars were checked daily and the date of the molt to the second instar and of pupation were recorded as was mortality. Caterpillars were transferred to new containers with food on 16 May, 31 May, and 9 June 1994.

Pupae were separated by sex based on size and morphology, weighed, and groups of approximately 5 male and 5 (2–6) female pupae from the same area, but not necessarily the same family, were placed in paper bags. When possible, males and females from the same area were used—but because the low-density populations produced fewer males than females, males from high-density sites were used in 8 of the 94 bags. Neither production or mass of the 15 egg masses in these groups differed from crosses made within site groups. The number of females and numbers of bags for the six areas is as follows (females, bags): high-density sites—Montague (72 females, 16 bags), Holyoke (54, 14), Ludlow (67, 17); low-density sites—Quabbin 2 (73 females, 17 bags), Quabbin 17 (81, 18), Quabbin 20 (50, 11). The bags were stapled shut, lightly sprayed with water every several days, and kept in the laboratory with variable day–night light cycles depending on the work patterns of researchers. Moths emerged, mated, and laid eggs on the inside of the bags. Egg masses were cut out of the bags and weighed.

Two types of accidental mortality occurred during rearing. Contamination of food cups with black mold occurred with one batch of diet and killed 67 individuals. Approximately half of these were from the Ludlow population. An additional 17 individuals from the

TABLE 2. Variation in development of male and female gypsy moth caterpillars from all areas reared in the laboratory. Data are means ± 1 SE; *N* = no. of caterpillars.

	Second instar		Pupation		
Sex	Time to reach (d)	*N*	Time to reach (d)	*N*	Pupal mass (g)
Male	8.0 ± 0.08	465	37.2 ± 0.18	467	0.67 ± 0.02
Female	7.5 ± 0.06	616	43.0 ± 0.15	622	1.89 ± 0.01
F†	20.8		630.8		3 587.4
df	1, 1 079		1, 1 089		1, 1 087

† *F* ratio compares values for the two sexes; in all cases $P < 0.01$.

Holyoke population were killed when a tray of containers was pushed against a heater. Individuals killed in these ways were not included in the analysis. Because the sexes differ, only development rates of individuals that survived to pupae and could be sexed were used. Sex ratios were based on pupae.

In the summer of 1994 pupae from field sites were collected and weighed for comparison to the laboratory-reared pupae.

Statistical analysis

The initial analysis was performed with a nested ANOVA (SYSTAT [Wilkinson et al. 1992] with corrected *F* values based on mean-squares of the density [df = 1], and area × density [df = 4]) to test for variation associated with density, high or low, of the sites. Mean values of families were used to test for variation among populations. One-way ANOVA was used to test for variation among sites apart from density. In cases where values for males and females differed, the sexes were analyzed independently or sex was used as a covariate.

RESULTS

Variation between the sexes

Males and females varied significantly in the time to the molt to the second instar, time to reach pupation, and pupal mass (Table 2). Further analyses consider the sexes separately or as a covariate.

Variation between tops and bottoms of egg masses

First-laid eggs may receive more yolk, and this could result in more vigorous caterpillars. Therefore, caterpillars were selected from the two halves for rearing. However, no significant differences occurred in the time to reach the second instar ($F = 0.53$, df = 1, 1343) time to pupation ($F = 0.01$, df = 1, 1087), or in pupal mass ($F = 0.19$, df = 1, 1086) among caterpillars from the tops and bottoms of egg masses based on ANOVA of sex, area, and egg mass half. Sex ratio was also the same for both halves of the egg masses (total proportion male for both halves for high-density sites was 0.52, and for low-density sites was 0.33 and 0.36 for the top and bottom halves, respectively). In further analyses, the data for caterpillars from tops and bottoms of egg masses were combined.

Variation among families within populations

Significant variation among families occurred for all populations in the time it took to reach second instar (Table 3). All but two populations, Quabbin 17 (low density) and Holyoke (high density), had significant variation among families in the time to pupation. All but two populations, Quabbin 17 and Quabbin 2 (low), had significant variation in pupal mass among families. This indicates that offspring of different females within a population can vary in ''quality'' or performance. This variation could be due to genetic and/or maternal effects.

TABLE 3. Results of ANOVA for variation among gypsy moth families within areas. *N* for Holyoke is 12 families and for all other areas is 10 families. See Tables 4 and 5 for actual values for all families. Sex is a covariate in all analyses.

	Development time								
	Second instar			Pupa			Pupal mass		
Area	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df
Low density									
Quabbin 17	2.7	.01	9, 167	1.2	.30	9, 168	1.2	.30	9, 168
Quabbin 2	3.5	.00	9, 166	3.0	.00	9, 168	1.6	.13	9, 168
Quabbin 20	6.0	.00	9, 151	2.8	.00	9, 151	2.4	.02	9, 151
High density									
Holyoke	3.8	.00	11, 156	5.0	.00	11, 158	3.1	.00	11, 158
Ludlow	2.8	.01	9, 139	1.4	.20	9, 139	2.4	.02	9, 139
Montague	2.7	.01	9, 178	2.3	.02	9, 181	2.7	.01	9, 181

TABLE 4. Variation in development of families of gypsy moth caterpillars hatching from egg masses collected from the field. All data are means ± 1 SE.

Area	Egg-mass length (cm)	Time to second instar (d)		Time to pupation (d)	
		Males	Females	Males	Females
Low density					
Quabbin 17	4.62^{a} ± 0.15	7.97 ± 0.28	7.45 ± 0.14	36.02 ± 0.26	42.28^{ab} ± 0.45
Quabbin 2	3.97^{a} ± 0.29	8.39 ± 0.29	7.74 ± 0.24	37.84 ± 0.76	43.49^{b} ± 0.51
Quabbin 20	4.13^{a} ± 0.31	7.33 ± 0.32	7.50 ± 0.24	37.18 ± 0.75	43.29^{ab} ± 0.50
High density					
Holyoke	2.00^{b} ± 0.06	8.27 ± 0.25	7.63 ± 0.40	37.85 ± 0.60	42.63^{ab} ± 0.64
Ludlow	2.50^{bc} ± 0.18	8.21 ± 0.29	7.32 ± 0.17	37.10 ± 0.55	42.06^{a} ± 0.50
Montague	2.95^{c} ± 0.15	7.86 ± 0.20	7.35 ± 0.25	37.09 ± 0.33	44.30^{b} ± 0.55

Notes: Values that share the same superscripted lowercase letter do not differ significantly. For egg masses, ANOVA: $F = 26.2$, $P < 0.001$, df = 5, 56). Variation in time to second instar was not significant for either sex, nor did time to pupation vary among sites for males. However, time to pupation did differ significantly for females ($F = 2.38$, $P = 0.05$, df = 5, 56) and Tukey's test showed that the Ludlow and Montague sites differed significantly.

To determine if any of the variation among families within populations was related to the size of the egg mass from which the caterpillars hatched, we tested for a correlation between the lengths of the egg masses and the performance of the caterpillars as indicated by time to reach second instar, time to pupation, and pupal mass. Sexes were considered separately. In only 2 of 36 comparisons was variation in a trait related to the length of the egg mass; both of these involved females from the high-density site at Montague. Mass of pupae and length of time to second instar were both positively correlated to the length of the egg mass ($r = 0.60$ and 0.63, respectively; $P < 0.05$, df = 9). These tests are not very robust because we had only 10 or 12 families for each population—but they revealed no strong relationships between the size of the initial egg mass and performance of caterpillars.

Variation between families from high- and low-density populations

We next analyzed the data to determine if characteristics of high- and low-density populations varied in a consistent manner when caterpillars were reared in the laboratory. The initial egg masses from the low-density populations were significantly larger than those from the high-density populations (Tables 1 and 4). This may indicate variation in the quality of ovipositing females in populations with different population histories. Comparisons of development time, pupal mass, survival, and sex ratio were done using family means, and relationships with density were tested with a nested analysis of variance.

We first consider development time. The ANOVA nested for density showed no difference in the time to reach the second instar for males ($F = 0.59$, df = 1, 4) or females ($F = 0.89$, df = 1, 4). Time to pupation did not vary significantly when nested for density for males ($F = 0.03$, df = 1, 4) or for females ($F = 0.0$, df = 1, 4) but did vary significantly between two of the high-density populations (Ludlow and Montague) (Table 4). Caterpillars from Quabbin 17 developed particularly quickly to the pupal stage, with both males and females reaching pupation on average 1–2 d faster than caterpillars in other populations. The egg masses from the Quabbin 17 population were the longest, but no consistent pattern between egg-mass length and development rate was apparent among all the populations.

Survival of caterpillars did not vary significantly with density of the maternal population when nested for density ($F = 0.18$, df = 1, 4), but did vary significantly among populations (Table 5). The lowest and highest means for survival occurred in two high-density populations.

The mean mass of pupae did not vary with density of populations with nested ANOVA (males: $F = 2.7$, df = 1, 4; females: $F = 0.0$, df = 1, 4) and no variation existed with simple one-way ANOVA (Table 5). Nested ANOVA showed no significant difference in mass of the egg masses produced by adults emerging from the pupae ($F = 0.5$, df = 1, 4), but one-way ANOVA indicated significant variation among populations. The number of egg masses varied among the populations (Table 5); the greatest number of egg masses was obtained from a low-density population (Quabbin 2) and the second greatest from a high-density population (Ludlow). This was not directly related to the number of females used to obtain egg masses. Pupae from caterpillars reared in the laboratory were heavier than those collected from the field sites in the summer of 1994 (Table 6). The pupae collected from the low-density sites at Quabbin were heavier than those from the high-density populations at Holyoke and Ludlow.

The only characteristic to vary consistently with the histories of the populations was sex ratio. Low-density populations had more females than high-density populations (Nested ANOVA: $F = 17.4$, $0.05 > P > 0.01$, df = 1, 4) (Table 5). This variation could not be explained by differential survival among the groups. A correlation analysis of survival and sex ratio for families across all areas was not significant ($r = 0.14$, N

TABLE 5. Measured parameters for gypsy moth families within populations. Data are means ± 1 SE; means followed by the same superscript lowercase letters are not significantly different according to Tukey's test. None of the means of egg-mass masses were significant with Tukey's test.

Area	Survival to pupation	Sex ratio† of pupae	Pupal masses (g)		Egg masses‡		
			Male	Female	Mass (g)	*N*	Fem.
Low density							
Quabbin 17	$0.78^{ab} \pm 0.03$	$0.34^{ac} \pm 0.03$	0.68 ± 0.01	1.90 ± 0.03	0.44 ± 0.03	29	81
Quabbin 2	$0.88^{ac} \pm 0.02$	$0.34^{ac} \pm 0.05$	0.66 ± 0.02	1.92 ± 0.05	0.53 ± 0.03	56	67
Quabbin 20	$0.77^{ab} \pm 0.04$	$0.27^{a} \pm 0.03$	0.65 ± 0.02	1.86 ± 0.07	0.41 ± 0.04	26	50
High density							
Holyoke	$0.74^{ab} \pm 0.03$	$0.61^{b} \pm 0.05$	0.66 ± 0.01	1.88 ± 0.07	0.48 ± 0.04	17	54
Ludlow	$0.89^{c} \pm 0.03$	$0.51^{bc} \pm 0.04$	0.70 ± 0.01	1.97 ± 0.04	0.54 ± 0.03	42	67
Montague	$0.88^{ac} \pm 0.02$	$0.47^{bc} \pm 0.04$	0.69 ± 0.02	1.83 ± 0.07	0.46 ± 0.04	27	72
F	6.02**	9.19***	1.37§	0.46‖	2.64*		
df	5, 56	5, 56	5, 56	5, 56	5, 191		

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; § $P = 0.25$, ‖ $P = 0.84$.
† Proportion male.
‡ *N* = no. of egg masses; Fem. = no. of females crossed to obtain eggs.

= 62). The frequency distribution of the sex ratios of families from the high-density populations suggests a weak bimodal distribution, with one group having ~30% males and another closer to 50–60% males (Fig. 1). While several families from low-density populations had ~50% males, most had between 15 and 35% males. No families produced all of one sex. Egg-mass length was not related to sex ratio in families from low-density areas, but in high-density areas sex ratio was correlated with the length of the egg mass (Fig. 2). Although there were three small egg masses from high-density areas that produced a high proportion of female caterpillars, other egg masses <3 cm from low- and high-density areas either produced sexes equally or produced a preponderance of males.

Variation in the rate of hatching of males and females has been noted previously for gypsy moths (Leonard 1968, Bell et al. 1981). We selected the first caterpillars to hatch from egg masses to ensure consistency in the time from hatch. Because the egg masses from the low-density populations were larger, we were more likely to obtain 13 caterpillars on the first day of hatching. For high-density populations with smaller egg masses, caterpillars were more frequently collected over 2–3 d (Table 7). We did not record the time to total hatch, but most caterpillars had hatched by the third day.

To determine if day of hatch influenced the sex ratio we compared the sex ratio of caterpillars collected on the first and second days of hatch for those groups in which not all caterpillars were collected on one day. We considered the top and bottom halves of each egg mass separately. Variation in the sex ratio of caterpillars hatching on first and second days was not significant for either the low-density or high-density populations although the data from Montague approached significance (chi-square = 3.66, $0.10 < P < 0.05$). In three populations the trend was for more females to hatch on the first day, but the Holyoke population had slightly more males hatching on the first day, and two low-density populations had very similar sex ratios on the two days. There was little variation between the sex ratios of caterpillars collected over two days and those for all families (Table 7).

Many more male than female pupae were collected from the low-density populations at Quabbin and the high-density population at Holyoke in the summer of 1994 (Table 6). The number of pupae collected from

TABLE 6. Pupal masses of gypsy moth pupae reared in these laboratory experiments and pupae collected from field sites in the summer of 1994. Data are means ± 1 SE except for Quabbin. *N* = no. of pupae.

Area	Laboratory		Field collected			
			Male		Female	
	Male pupal mass (g)	Female pupal mass (g)	Pupal mass (g)	*N*	Pupal mass (g)	*N*
Quabbin†	0.65 − 0.68	1.86 − 1.90	0.45 ± 0.02	23	1.40 ± 0.25	4
Holyoke	0.66 ± 0.01	1.88 ± 0.07	0.39 ± 0.01	51	1.13 ± 0.11	7
Ludlow	0.70 ± 0.01	1.97 ± 0.04	0.40 ± 0.06	6	0.85 ± 0.06	4

† For the low-density Quabbin sites, a range is given for the laboratory-reared pupae since the three means from the three Quabbin sites were very similar; the range is that of the three means.

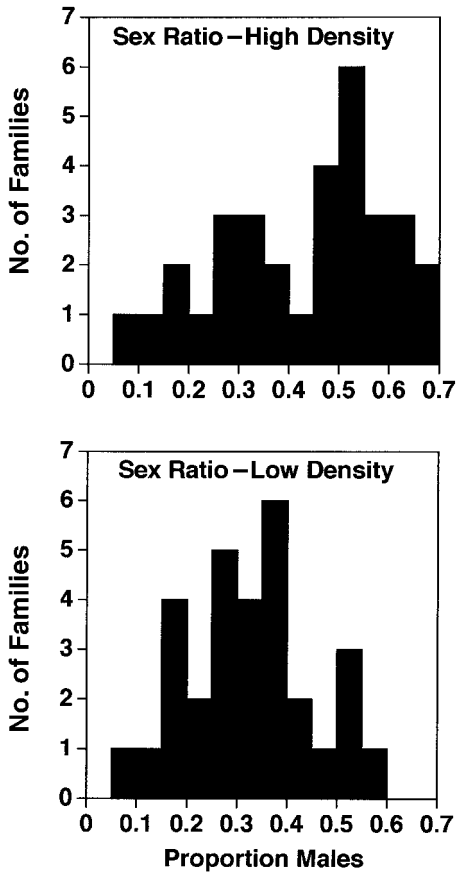

FIG. 1. Frequency distribution of the sex ratio (proportion males) of families of gypsy moths from high- and low-density areas in western Massachusetts.

Ludlow was smaller and more equitable between the sexes.

DISCUSSION

We have found considerable variation among families in the performance of gypsy moths when caterpillars are reared in a controlled environment and fed artificial diet. However, we found little support for the hypothesis that performance of gypsy moths varies in a consistent manner between high- and low-density populations when caterpillars are reared in optimal, controlled conditions. This study does not provide evidence for changes in population quality of gypsy moth being carried over to the next generation by maternal influences.

Eggs used in these experiments were treated with formalin to remove disease contamination, and no deaths caused by disease were identified. Therefore, elimination of disease is one difference between the laboratory populations and field populations. Other differences are lack of both crowding and potential variation in food quality. The masses of pupae collected from the field in 1994 indicate that the conditions for caterpillars in the laboratory were superior to those in the field (Table 6). Pupal masses and sizes of egg masses both indicate that the density of caterpillars can influence the fecundity of moths in field populations. However, these effects do not seem to be carried over to the next generation under ''ideal'' conditions.

Our results conflict with those of Rossiter (1991*a*) who found that offspring attained higher pupal mass if their mothers fed on trees with high damage to the leaves, and that the development rate of daughters varied with parental diet. Rossiter's moths were from a population initially collected from Connecticut and reared in the laboratory for three generations. The masses of female pupae in her studies were between 3 and 4 g, while the average mass of female pupae in our experiments were <2 g even though we used the same temperature. Rossiter only used the first five males and first five females to reach pupation in her analysis while we used all individuals reaching the pupal stage. There may be differences among individuals developing rapidly and slowly, which would influence Rossiter's results. She did not record sex ratio.

Lance et al. (1986) also reared gypsy moth caterpillars from eggs collected from low-density, increasing, and collapsing populations. They found no differences in development time for males, but females from the low-density population developed significantly more rapidly. The pupal mass of males varied among the different populations with the heaviest pupae being those from a population that was just beginning to increase and the smallest in one of two collapsing populations.

In our study the sex ratio of pupae varied strikingly between low- and high-density populations. That sex ratio of field populations of gypsy moths may vary with density has been previously noted. Campbell (1963*a*, *b*) attributed variation in sex ratios of field populations to disease and desiccation causing greater mortality among females, which have six instars and therefore are exposed to food limitation and mortality to a greater

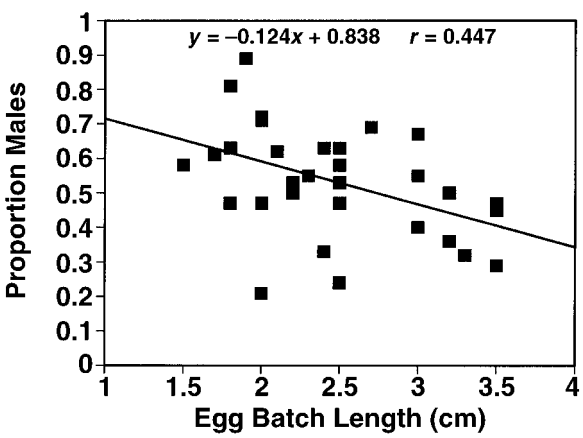

FIG. 2. Regression of sex ratio (proportion males) on length of the egg mass for families of gypsy moths from three high-density areas in western Massachusetts.

TABLE 7. Proportion of gypsy moth males hatching on days 1 and 2 for families in which caterpillars were collected for two days, proportion males for both days, proportion males for all families, and proportion of males among pupae collected from three areas in the summer of 1994. *N* = no. of pupae; all pupae collected from the Quabbin sites in 1994 were combined in one sample.

Area	Proportion males					
	Hatching on day 1	Hatching on day 2	Combined hatching	All families	Field pupae	*N*
Low density						
Quabbin 17	.50	.50 (1)	.50	.34		
Quabbin 2	.32	.30 (7)	.31	.34	.85	27
Quabbin 20	.25	.35 (2)	.30	.33		
High density						
Holyoke	.73	.59 (12)	.65	.61	.88	58
Ludlow	.42	.52 (9)	.47	.51	.40	10
Montague	.36	.56 (7)	.46	.47	···	···

Notes: The number in parentheses is the no. of families out of 10 families (for Holyoke, out of 12 families) for which caterpillars were collected for 2 d. There was no field collection of pupae at Montague.

extent than males, which have only five larval instars. Campbell (1967) found no variation in the sex ratio of caterpillars hatching from eggs or among those collected as first instars from field populations. Leonard (1968) found that sex ratio varied with day of hatching with females hatching first. He also observed variation in sex ratio of caterpillars hatching from different egg masses. Bell et al. (1981) studied the sex ratio of caterpillars hatching from egg masses from three different populations—two field and one laboratory population. They also observed more males among caterpillars from a high-density population that was infected with virus than among caterpillars from a low-density population lacking virus. However, in their study the sex ratio of the low-density population was approximately equal, and the high-density population had >50% males. In our study the low-density populations had ~33% males and the high-density populations had ~50% males. This difference might be explained by our collecting caterpillars from the first eggs to hatch, which could have biased the results for both groups toward lower sex ratios. While sample sizes were small, the sex ratio of pupae from the innocuous population studied by Lance et al. (1986) favored females, from the release population was equal, and from the collapsing populations favored males. These trends in sex ratio and density are similar to ours, which suggests that the association is robust.

The impact of sex-ratio elevation in high-density populations could greatly affect the potential rate of increase of gypsy moth populations. Not only are egg masses from high-density populations smaller, but the reduction in the proportion of females further reduces the reproductive potential. By multiplying the estimated number of eggs per mass for high- and low-density populations by the sex ratio we find that the three low-density populations produced on average 418, 508, and 582 females per egg mass while the high-density populations produced on average 84, 161, and 183 females per egg mass. Therefore the production of daughters per family is potentially 2 to 6 times greater in low-density populations. This effect could have a strong influence on the dynamics of populations.

We do not know what mechanism could cause this sex ratio distortion. Because the investment in daughters and sons by gypsy moth parents appears to be equal (i.e., no parental care), an equal sex ratio is expected. The high-density populations fit this expectation but the low-density populations deviate from it. It is possible that the sex ratios of caterpillars from both high- and low-density areas would have been higher if all the eggs had been hatched. This is unlikely though since a high proportion of the eggs usually hatched in the first day or two. The resources available for yolk provisioning of eggs could vary with population density and this might relate to the sex ratio of eggs. However, sex ratio was similar between the first- and last-laid eggs in the egg masses, which might argue against an association between yolk and sex ratio. We do not know of a mechanism for how yolk provisioning could influence the sex ratio.

Pupae collected from field populations in 1994 were strongly biased in favor of males at two sites, but the two sexes were more equally represented at one high-density area. It is possible that the technique used for monitoring pupae by attaching burlap strips to tree trunks might favor collection of male pupae.

A relationship between sex ratio and density has been found in other studies. Mauffette and Jobin (1985) compared the sex ratio of pupae collected from 14 different sites in Quebec and found a highly significant correlation between the proportion of male pupae and population density. High-density populations had 55–60% males and low-density populations had ~40% males. Similarly, Fuester and Taylor (1996) studied field populations of gypsy moth pupae over 9 yr and

found a highly significant relationship of sex ratio with density. High-density populations had ~60% males and low-density populations had ~45% males. The change between these two extremes was linear over time. If the sex-ratio deviation had a genetic basis, a gradual change of this sort would be expected. Fuester and Taylor (1996) also recorded higher mortality of female pupae in high-density years, and if a similar trend occurred among larvae this would accentuate the sex-ratio deviation. Neither of these two studies measured the primary sex ratio, but their results are similar to ours, with low-density populations having more females than high density populations.

One factor that could select for a change in the sex ratio from equality is local mate competition or inbreeding (Hamilton 1967). Gypsy moths lay eggs in masses and adult females do not fly. Therefore, when density is low it is likely that brothers and sisters will have an increased probability of mating. Inbreeding could select for increased production of daughters, particularly if males are able to mate more than once. This would require a genetic basis to the determination of sex ratio.

Unfortunately we did not have the resources to rear caterpillars from the eggs to determine if sex ratio modification is environmentally determined or if it would be expressed in the next generation in a consistent way, which would indicate a maternally or genetically determined characteristic. It is possible that sex ratio and pupal and egg-mass size are immediately restored when caterpillars are reared in the absence of disease, on adequate diet, and in uncrowded conditions. Our observation that sex ratio is related to the size of the egg mass in high-density populations (Fig. 2) might suggest an environmental rather than a genetic basis for the variation recorded here.

We conclude that the small egg-mass sizes of field populations at high density are associated with environmental conditions such as crowding, disease, food limitation, or food-quality variation, but that fecundity is restored without a lag when caterpillars are reared under laboratory conditions. Development time and pupal mass did vary significantly among families within populations in our study, but variation was not related to the size of the initial egg mass, which is the only characteristic we had that reflects the phenotype of the mother. Therefore, although maternal effects or qualitative variations among families are expressed under controlled conditions, no pattern of variation with population density other than sex ratio was apparent.

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