DENSITY-DEPENDENT SUPPRESSION OF EXPERIMENTALLY CREATED GYPSY MOTH, *LYMANTRIA DISPAR* (LEPIDOPTERA: LYMANTRIIDAE), POPULATIONS BY NATURAL ENEMIES

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SUMMARY

1. Experimental manipulations of densities of gypsy moths revealed a strong, positive spatially density-dependent reduction in population size, a response not evident in past studies of natural populations in North America.

2. Positive density-dependent mortality occurred during the early and mid larval stages and was primarily due to *Compsilura concinnata*, a polyphagous parasitoid.

3. The oviposition rate of *Parasetigena silvestris*, an oligophagous parasitoid of gypsy moths, was initially inversely density-dependent but became positively density-dependent during the late larval period.

4. *Phobocampe disparis* showed an inversely density-dependent response, and predation by small mammals on pupae deployed in the litter was lower in plots with higher numbers of pupae.

5. We conclude that if gypsy moth population densities fluctuate asynchronously on a spatial scale of a few hectares, the density-dependent responses of *C. concinnata* and *P. silvestris* could suppress the populations to a point where small mammal predation would be able to prevent population increase. This phenomenon may explain the apparent stability of gypsy moth populations on a region-wide basis for the many years between outbreaks.

INTRODUCTION

In the north-eastern United States the gypsy moth, *Lymantria dispar* (L) (Lepidoptera: Lymantriidae), is one of the most damaging forest insect pests. Larvae hatch in April or May, climb to the tops of trees and often disperse on the wind. They remain in the forest canopy until they moult to the fourth instar. In low to moderate density populations, late instars (4–6) migrate daily from night-time feeding sites to protected daytime resting sites under bark flaps or in the litter on the forest floor. This behaviour may have evolved in Europe in response to parasitism by tachinid flies and predation by insectivorous birds...
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(Campbell & Sloan 1976), but in North America it leads to high rates of predation by small mammals, particularly *Peromyscus leucopus* Raf. Larvae pupate in their resting sites and adults emerge in July. Females do not fly, and they deposit their eggs in a single mass not far from the site of pupation.

In North America, gypsy moths in most forest stands remain at low densities for many years and then erupt into an outbreak phase which may last for several years. The major goal of research on gypsy moth population dynamics is to identify those factors or agents responsible for maintaining populations at low densities and the mechanisms for release to outbreak levels. It has been suggested (Campbell 1976; Campbell & Sloan 1977, 1978; Campbell, Sloan & Biazak 1977) that predation by small mammals on late instars and pupae maintains populations at low densities and that this predation is positively density-dependent (Campbell, Sloan & Biazak 1977). There are also eight introduced parasitoids established in North America as well as ten endemic parasitoid species that attack gypsy moths. While there have been reports of relatively high levels of parasitism by some gypsy moth parasitoids (Campbell & Podgwaite 1971; Doane 1971; Barbosa, Capinera & Harrington 1975; Blumenthal, Fusco & Reardon 1979; ODell & Godwin 1979; J. R. Gould & J. S. Elkinton, unpublished), most researchers (e.g. Reardon 1976; Campbell, Sloan & Biazak 1977; Ticehurst et al. 1978) believe that parasitoids do not cause sufficient mortality to limit the growth of gypsy moth populations. The total amount of mortality caused by a given agent is not as important to population regulation, however, as the density dependence of the response. The strength of the density-dependent response over a range of host densities determines whether the host population is stabilized, goes through regular cycles, or exhibits chaotic behaviour (May 1986).

There is as yet no strong evidence that small mammals cause positive density-dependent mortality in populations of gypsy moths. In fact, there is some evidence (Elkinton et al. 1989) that this predation is inversely density-dependent. Some researchers have found positive density-dependent mortality due to parasitoids (Reardon 1976; Sisoevic 1977; ODell & Godwin 1979; Furuta 1982) and others have found a negative correlation between percentage parasitism and host density and/or percentage defoliation (Weseloh 1973; Reardon 1976; Reardon & Podgwaite 1976; Ticehurst et al. 1978). In many of these studies, however, the methods used to determine host density or percentage parasitism were unsatisfactory (Gould et al. 1989).

Density dependence may be difficult to detect from traditional temporal life-table studies. Natural stochastic variation may obscure underlying density-dependent processes rendering them difficult to detect (Hassell 1985, 1987), although Dempster & Pollard (1986) and Mountford (1988) have disputed this contention. Also, life-table studies which look at average mortality occurring in populations over several generations may not detect density-dependent responses to spatial heterogeneity among subpopulations within a generation (Hassell 1987; Hassell, Southwood & Reader 1987). Furthermore, when natural population densities are close to an equilibrium, there may be no direct density-dependent mortality of the host population (Murdoch & Reeve 1987). It has been suggested (Hassell 1985, 1987; Gaston & Lawton 1987; Murdoch & Reeve 1987) that a solution to these problems is to manipulate population densities (e.g. Furuta 1976, Karieva 1985; Reeve & Murdoch 1985) rather than to rely on life-table data collected from natural populations over several generations. Our study was designed to manipulate the density of gypsy moth larvae to determine if natural enemies could respond to local increases in density in a density-dependent manner.
MATERIALS AND METHODS

Site description and preliminary counts of egg masses

Our study was conducted in Cadwell Memorial Forest in Pelham and Belchertown, Massachusetts during 1987. We established eight 1-ha plots (100 m × 100 m) in the forest, spaced at least 750 m from one another. Oaks (Quercus rubra L., Q. velutina Lam. and Q. alba L.) were the predominant overstorey trees in all plots, which also contained Acer rubrum L. and to a lesser extent A. saccharum Marsh, Betula lenta L., B. lutea Michx., B. populifolia March and Fraxinus americana L.

In each plot we established a 10 × 10 grid of points, with 10 m between points, to serve as a framework for various sampling regimes. Prior to density manipulations we conducted an egg-mass survey in each plot by counting all egg masses within a circle of 7.5 m radius around five egg-mass sampling points. Mount Lincoln in Caldwell Forest has a history of outbreaks and had defoliating gypsy moth populations during an outbreak from 1979 to 1981. Following the population crash in 1981 densities remained very low and the pre-season egg-mass counts revealed no egg masses in any of the plots.

Release of larvae

Egg masses for the release experiment were collected in an area with expanding, moderately high density gypsy moth populations in Hopeville, Connecticut. These egg masses were submerged for 1 h in a 10% formalin solution to remove viable nuclear polyhedrosis virus (NPV) from the egg surface (Bell et al. 1981) and were then rinsed for 1 h with water. The eggs were divided into four groups of different sizes (two replicates per group size) to give us four densities of larvae at hatch. We weighed each group and then placed the eggs in 100 7.5 x 10 cm screen packets (200 packets for the highest density). We then sampled one of the largest packets and determined the number of larvae that emerged. The resulting value of number of larvae emerging g⁻¹ of eggs was multiplied by the weight of all eggs for each group to estimate the number of larvae at hatch (see Table 1).

On 4 May 1987 we released larvae at densities that corresponded to that expected from 174 to 4600 egg masses ha⁻¹, assuming a hatch of 250 larvae per egg mass. Egg-mass densities above 2500 ha⁻¹ would be expected to result in complete defoliation of a forest stand (Wilson & Talerico 1981). Prior to the release we had collected eighteen egg masses from a high density site in Hardwick, Massachusetts (20 km from Cadwell Forest). These

<table>
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<th>Density class</th>
<th>Plot</th>
<th>Number of larvae ha⁻¹ at hatch</th>
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</thead>
<tbody>
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<td>1</td>
<td>1A</td>
<td>59,304 (± 2206)</td>
</tr>
<tr>
<td>1</td>
<td>1B</td>
<td>43,538 (± 1619)</td>
</tr>
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<td>2</td>
<td>2A</td>
<td>146,825 (± 5461)</td>
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<td>2</td>
<td>2B</td>
<td>81,821 (± 3043)</td>
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<tr>
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<td>3A</td>
<td>374,542 (± 13,930)</td>
</tr>
<tr>
<td>3</td>
<td>3B</td>
<td>296,157 (± 11,015)</td>
</tr>
<tr>
<td>4</td>
<td>4A</td>
<td>1143,112 (± 42,516)</td>
</tr>
<tr>
<td>4</td>
<td>4B</td>
<td>1144,325 (± 42,561)</td>
</tr>
</tbody>
</table>
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egg masses were placed individually in 30-ml plastic cups and were kept shaded in Cadwell Forest. Hatch was monitored daily and we timed our release to coincide with that observed from these egg masses. Oak trees in all plots were beginning to expand their leaves at this time.

To ensure that larvae were distributed throughout each plot we released them at 100 points in the 10 x 10 grid. We deployed egg masses by stapling the screen packets to the trunks of the trees closest to the release points at a height of 1.5 m. Emerging neonates passed easily through the mesh of the screen.

Monitoring dispersal of larvae

To monitor dispersal of larvae away from the plots we established eight transect lines (two per cardinal direction) extending 150 m away from plots 4A and 4B (the plots with the highest density of released larvae). Transect lines were 25 m from the corners of the plots and were 50 m apart. We wrapped a burlap band 24.5 cm wide at a height of 1.5 m around every oak tree within 5 m of either side of the transect line. When larvae were in the fifth and sixth instars we counted the numbers of live and dead larvae under each burlap band to determine how far from the plots the larvae dispersed and if the magnitude of mortality changed with distance from the plot. We regressed both the log10 of the total number of larvae m-1 of burlap and the arcsine of the square root of the proportion of dead larvae on the distance from the plot.

Monitoring changes in density

We monitored the change in density of gypsy moths in all plots throughout the season by taking weekly density estimates. Frass traps (Liebhold & Elkinton 1988a, b) were deployed at forty-eight points in each plot to estimate the densities of third to fifth instar larvae. Each week we counted the number of frass pellets collected overnight in the 50-cm diameter funnel-shaped traps. We also collected twenty larvae from each of the four higher density plots and held them individually in 360 ml cups with oak leaves during the period we measured frass drop. By dividing the number of pellets collected in the total area covered by the frass traps by the number of pellets produced per individual larva we were able to estimate the number of larvae ha-1 (see Liebhold & Elkinton 1988a, b for a complete description of this method).

After 2 July when larval densities became so low that estimates from frass traps were no longer accurate, we measured changes in density by counting the number of larvae and pupae m-1 of burlap band each week. There were four sampling points per plot and the twenty-five trees closest to each point were wrapped with a strip of burlap 24.5 cm wide. Several vertical slits were cut in each band to create flaps which larvae used as daytime resting sites. It should be noted that it is not possible directly to convert the number of larvae under burlap bands to number ha-1, and we therefore used these measures to estimate the relative drops in density occurring during the late instars. Mortality of larvae under burlap bands may differ from that experienced elsewhere in the population, but at the low densities experienced this method was our only option.

At the end of the season we estimated the final egg-mass density, using the same five sampling points used for the pre-season egg-mass count. These points were situated at least 25 m from the nearest burlap point because burlap bands can influence pupal survival (Bess, Spurr & Littlefield 1947; Campbell, Hubbard & Sloan 1975). We recorded the location of these egg masses and returned following hatch in the spring to determine the number of larvae present in the next generation. The lengths of the egg masses were
measured and the regression of Moore & Jones (1987) was used to estimate the number of eggs per mass (newly hatched larvae consume the chorion of the egg, therefore eggs from which larvae had emerged could not be counted). We then counted the number of unhatched or parasitized eggs in each mass and subtracted this value from the number of eggs to estimate the number of larvae.

Monitoring larval mortality

We monitored larval mortality due to parasitoids and disease by collecting 100 larvae per plot each week. Four grid points were selected at random in each plot on each sampling occasion. Early instars, which were mainly in the canopy, were sampled by climbing the oak tree closest to the sampling point using climbing ropes. Branches were cut with pole pruners, dropped onto plastic tarps, and larvae were collected. We also collected larvae from the litter and understory vegetation around the sampling point. When the larvae reached the fourth instar and descended from the canopy during the day we deployed burlap bands on the five trees closest to the randomly selected sampling points on the day prior to sampling. Larvae were collected from under the burlap bands, which were then removed, as well as from the litter and the understory. On each sampling occasion we also recorded the number of larvae bearing large macrotype eggs laid by *Parasitigena silvestris* Robineau-Desvoidy (Diptera: Tachinidae).

Larvae were placed individually in 30-ml plastic cups containing an artificial diet (Bell *et al.* 1981) and were reared in an outdoor screen cage located approximately 200 m from one of the plots. The larvae were checked once a week until death or adult emergence and puparia or cocoons of emerging parasitoids were keyed to species (Simons, Reardon & Ticehurst 1979). Dead larvae, from which no parasitoid emerged, were checked for the presence of the polyinclusion bodies of NPV using a phase contrast microscope at 1000 x magnification, and for immature parasitoids that failed to emerge. If we failed to detect polyinclusion bodies or parasitoids, we classified the mortality as ‘unexplained’.

Because predation on released larvae could not be measured directly, we conducted additional observations to assess the potential impact of avian predators. When larvae were in the second and third instars, and were located mainly in the canopies of the trees, we attempted to determine whether foliage-gleaning birds were attracted to areas with high density gypsy moth populations. Four sampling points were chosen in the two plots with the highest densities. We also established a control plot 100 m from each test plot that had a similar forest composition and the same configuration of sampling points, but few gypsy moths. Two experienced ornithologists recorded the numbers of each species of foliage-gleaning birds that were heard or seen over an 8-min period at each sampling point. Sampling was conducted between 06.00 and 09.00 hours from 29 May to 2 June with each ornithologist observing the birds in one plot and the adjacent control plot. The plots were sampled in a different order on each day.

Monitoring pupal mortality

We attempted to quantify mortality of pupae due to predators and parasitoids. In all eight plots 150 male and fifty female laboratory-reared pupae (from Otis Methods Development Center; New Jersey strain, generation 30) were attached individually to small pieces of burlap with beeswax. Fifty of the male pupae were placed in wire mesh cages measuring 44 × 10 × 5 cm (1.27 cm mesh) to exclude small mammals. We placed one uncaged male pupa and either an uncaged female pupa or a caged male pupa at each grid point. Pupae were placed on the ground at the base of the trees nearest to the grid point.
and were covered with leaf litter. They were checked on each of the next 3 days to determine survival. The significance of the differences in the survival of pupae between plots was calculated using the SPSS 9-0 survival procedure (Hull & Nie 1981) and the Lee–Desu D statistic (Lee & Desu 1972). After 3 days all surviving female pupae were returned to the laboratory and reared in 30-ml plastic cups to determine whether they had been attacked by pupal parasitoids.

**Analysis of density dependence and calculation of k-values**

To determine whether mortality occurring over the entire season and during four periods of time was density-dependent, we used the method of Varley, Gradwell & Hassell (1973). We estimated the killing power, $k$, of a mortality agent or agents which is defined as $k = \log_{10}(N_i/S)$, where $N_i$ is the density of the initial population and $S$ is the density of survivors after the action of the mortality agent(s). The $k$-value was regressed on the logarithm of the initial density ($N_i$). A regression line with a slope significantly greater than zero indicated positive density dependence, whereas inverse density dependence was indicated by a negative slope. In our study we recorded spatial density dependence in contrast to the temporal density dependence measured by Varley & Gradwell (1968). The values for $N_i$ and $S$ used to calculate the $k$-values for the entire season and for four periods of time are given in Table 2. We added 1.0 to all final density estimates because the logarithm of zero is undefined and most plots had an estimated density of zero egg masses ha$^{-1}$ at the end of the generation. The $k$-value for period 4 (which included mortality of pupae and adults and effects of adult sex ratio) was determined by subtracting $k_1$, $k_2$ and $k_3$ from $K$, the $k$-value for the entire season.

Several investigators have addressed statistical problems with detecting density dependence by regressing $k$-values on $\log_{10}N_i$ (e.g. Eberhardt 1970; Benson 1973; Slade 1977; Royama 1981a, b). One problem with this technique is that when regressing $k = \log_{10}(N_i/S)$ on $\log_{10}N_i$, $N_i$ appears in calculations of both the dependent and independent variables. A regression of this $k$-value on $\log_{10}N_i$ might result in a spurious positive relationship between the two variables (Atchley, Gaskins & Anderson 1976). Furthermore, the technique violates the regression assumption that all the error resides with measurement of the dependent variable. Varley & Gradwell (1968) developed a method to verify density dependence that solves both of these problems but their method is highly conservative (Slade 1977; Hassell, Southwood & Reader 1987). Other methods have been developed to overcome problems of detecting density dependence in series of annual censuses (i.e. Bulmer 1975; Pollard, Lakhani & Rothery 1987) but our study was

<table>
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<th>Period</th>
<th>Date</th>
<th>Stages</th>
<th>$k$-value</th>
<th>$N_i$</th>
<th>$S$</th>
</tr>
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<tbody>
<tr>
<td>Entire</td>
<td>4 May–7 Aug.</td>
<td>L$_1$–adult</td>
<td>$K$</td>
<td>No. hatching ha$^{-1}$</td>
<td>No. egg masses ha$^{-1} + 1$</td>
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<td>4 May–4 June</td>
<td>L$_1$-L$_3$</td>
<td>$k_1$</td>
<td>No. hatching ha$^{-1}$</td>
<td>No. larvae ha$^{-1}$ on 5 June</td>
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<td>2</td>
<td>5 June–2 July</td>
<td>L$_3$–L$_5$</td>
<td>$k_2$</td>
<td>No. larvae ha$^{-1}$ on 5 June</td>
<td>No. larvae ha$^{-1}$ on 2 July</td>
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<td>3</td>
<td>3 July–16 July</td>
<td>L$_5$–pupae</td>
<td>$k_3$</td>
<td>No. m$^{-1}$ burlap on 3 July</td>
<td>No. m$^{-1}$ burlap on 16 July</td>
</tr>
<tr>
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<td>17 July–7 Aug.</td>
<td>Pupae–adult</td>
<td>$k_4$</td>
<td>$K-k_1-k_2-k_3$</td>
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</table>

*L* = larval instar, $N_i$ = initial density, and $S$ = survivors.
limited to density dependence occurring within one generation. Following the suggestion of Hassell, Southwood & Reader (1987) we verified density dependence by regressing $\log_{10} S$ on $\log_{10} N_i$ using the regression technique of Bartlett (1949), which allows regression when there is error associated with both the dependent and independent variables. Reported probability values were obtained by determining the largest confidence interval around the slope that did not overlap with unity.

In contrast to the more typical use of stage-specific $k$-values, we calculated time-specific $k$-values for individual mortality agents across larval instars. We calculated weekly $k$-values for each parasitoid and for disease following a scheme derived from Royama (1981b). The weekly $k$-value for parasitoid $A$, $k_A$, can be defined as

$$k_A = -1 \cdot 0 \log_{10}(1 - m_A)$$

where $m_A$ is the proportion of hosts attacked and killed by parasitoid $A$ over a weekly interval in the absence of other simultaneous mortality agents. This value is what Royama (1981b) calls the marginal probability of mortality from a given agent. This value is greater than the proportion that are observed to die in rearings from parasitoid $A$, $v_A$, because a certain proportion of the larvae that would have died from parasitoid $A$ died instead from other parasitoids or disease. To calculate the marginal probability for each parasitoid ($m_A$) we solved eqns 12 and 13 in Royama (1981b) for the case of two simultaneous agents (Appendix 1).

These calculations produced estimates of $k$ for each parasitoid and disease for each week, and these values were summed up to a value that was equal (within c. 1%) to the $k$-value for all parasitoids and diseases for the week. $K$-values for each individual mortality agent were summed across weeks to yield a total for each parasitoid or disease for each period. The difference between the total $k$-value for the period (from density estimates) and the $k$-value of parasitism and disease (from rearings) constituted a measure of residual mortality (which includes predation). Individual $k$-values for each mortality agent for each period were regressed on the $\log_{10}$ of the density at the beginning of the period using the technique of Bartlett (1949). If the linear regression was significant, we also looked at the significance of quadratic and cubic trends in the data using sequential sums of squares.

_Parasetigena silvestris_ attacks middle and late instar larvae and emerges just prior to host pupation (T. M. ODell, personal communication). By the end of the fifth and sixth instars, when one would expect the parasitoids to emerge, host density was so low that we were unable to collect many larvae and thus directly measure mortality due to _P. silvestris_. We had a direct measure of the oviposition rate of this parasitoid, however, from our estimates of the number of larvae carrying macrotype eggs when larvae were still sufficiently numerous to sample. These eggs remain on the integument until a moult. During the sampling period gypsy moth larvae were advancing approximately one instar per week and we were therefore confident that the eggs seen on each sampling occasion had been deposited within the preceding week. For the analysis we regressed the $k$-values of _P. silvestris_ oviposition rates for each week on the larval density prior to the sampling occasion, based on frass trap density estimates, using the technique of Bartlett (1949).

RESULTS

The densities of gypsy moths in all eight plots were reduced between the first instar and adult stages to very low levels (Fig. 1) and at the end of the season there were more egg
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masses (surviving females) ha\(^{-1}\) in the lower density release plots (Fig. 1a, b) than in higher density release plots (Fig. 1c, d). Also, in all plots there were fewer larvae hatching in 1988 than were released in 1987, indicating that populations declined in density between generations following our release.

K-values for each plot over the entire season and the contributions to these values during periods 2 and 3 by the various mortality agents are shown in Table 3. Overall mortality was strongly density-dependent (Fig. 2). Positive density dependence occurred during periods 1 (instars 1–3; Fig. 3a) and 2 (instars 3–5; Fig. 3b), but regressions were not significant for periods 3 (instar 5–pupae; Fig. 3c) and 4 (pupae–adults; Fig. 3d). By regressing log\(_{10}\) S on log\(_{10}\) N, we verified that the relationships observed during periods 1 and 2 were density-dependent (P = 0.004 for period 1 and P = 0.019 for period 2).

No parasitoids emerged from larvae collected during period 1 so the drop in density during this period was presumably due to predation and/or dispersal of first instars. Foliage-gleaning birds, which might consume small larvae, were no more abundant in plots with high gypsy moth density than in control plots with no gypsy moths (Table 4). In fact, more birds were observed in the control plots. Larvae did disperse from the plots, and while we did not directly measure dispersal of first instar larvae we did find fifth and sixth instar larvae under burlap bands up to 130 m from plots 4A and 4B. No gypsy moths were found under burlap bands between 130 and 150 m from the plots.

During period 2, when the gypsy moths were mainly instars 3–5, there was high weekly mortality due to parasitoids (Fig. 4). *Compsilura concinnata* Meigen (Diptera: Tachinidae) caused the most parasitism, and there was some parasitism by *Cotesia melanoscela*
Table 3. K-values for mortality occurring over the entire season (K), from 4 May to 4 June (k1), from 5 June to 2 July (k2), from 3 July to 16 July (k3), and from 17 July to 7 August (k4) for individual mortality agents in eight plots in Cadwell Memorial Forest in 1987*

<table>
<thead>
<tr>
<th>Plot</th>
<th>1A</th>
<th>1B</th>
<th>2A</th>
<th>2B</th>
<th>3A</th>
<th>3B</th>
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<td>K</td>
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<td></td>
</tr>
<tr>
<td>kunex</td>
<td>0.22</td>
<td>0.20</td>
<td>0.23</td>
<td>0.09</td>
<td>0.40</td>
<td>0.15</td>
<td>0.26</td>
<td>0.95</td>
</tr>
<tr>
<td>kresid</td>
<td>-0.23</td>
<td>0.01</td>
<td>0.02</td>
<td>0.08</td>
<td>0.27</td>
<td>0.46</td>
<td>-0.31</td>
<td>-0.01</td>
</tr>
<tr>
<td>k4</td>
<td>3.74</td>
<td>1.70</td>
<td>0.75</td>
<td>2.17</td>
<td>3.08</td>
<td>3.16</td>
<td>3.57</td>
<td>2.73</td>
</tr>
</tbody>
</table>

*comp = C. concinnata, cote = C. melanoscelus, pdis = P. disparis, psil = P. silvestris, unex = unexplained mortality observed during rearing, and resid = residual mortality not observed during rearing. \( K = -10 \log_{10}(N_i/S) \) where \( N_i \) = initial density and \( S \) = number of survivors.

Fig. 2. Regression of \( K \) (the k-value for the entire season) on \( \log_{10} N_i \) (the number of gypsy moths ha\(^{-1}\) at hatch). '2' next to a data point indicates two observations. \( P = 0.012; \) slope = 1.86.
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Ratzeburg (Hymenoptera: Braconidae), Phobocampe disparis Veireck (Hymenoptera: Ichneumonidae) and P. silvestris. Unexplained mortality was also high, which is consistent with studies of other gypsy moth populations (Campbell 1963; Reardon & Podgwaite 1976; Blumenthal, Fusco & Reardon 1979). Unexplained mortality could be due to trauma associated with collection and rearing but parasitoid-induced mortality that does not result in the emergence of a parasitoid can be high (Blumenthal, Fusco & Reardon 1979; Godwin & ODell 1984). It is possible, therefore, that the overall contribution of parasitoids to the mortality observed in our populations was greater than the observed percentage parasitism values would indicate. Unexplained mortality could also be due to pathogens such as Streptococcus faecalis Doane (Doane 1970a).

Compsilura concinnata was the principal source of the density-dependent decline of the population during period 2 (Fig. 5a). The regression of $k_{comp}$ on $\log_{10} N_i$ was highly significant and had a relatively large positive slope. The response was non-linear, however, and levelled off above approximately 100,000 hosts ha$^{-1}$. A quadratic model ($Y = -18.87 + 7.32 X - 0.68 X^2$) fits the data better than the linear model ($P = 0.006$, $F = 21.77$, d.f. = 1, 5; and is shown in Fig. 5a). Unexplained mortality also increased significantly with increasing density (Fig. 5e) which suggests that it may be related to
TABLE 4. Total number of foliage-gleaning birds sighted or heard during 8-min periods at four sampling points from 29 May 1988 to 2 June 1988 in plots 4A and 4B and two control plots

<table>
<thead>
<tr>
<th>Specific Name</th>
<th>Common name</th>
<th>4A</th>
<th>Control</th>
<th>4B</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parus bicolor</td>
<td>Tufted titmouse</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dumetella carolinensis</td>
<td>Gray catbird†‡§</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Catharus fuscescens</td>
<td>Veery</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Catharus guttatus</td>
<td>Hermit thrush‡</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Vireo olivaceus</td>
<td>Red-eyed vireo†‡</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Myiobius varia</td>
<td>Black-and-white warbler‡</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Wilsonia canadensis</td>
<td>Canada warbler‡</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dendroica caerulescens</td>
<td>Black-throated blue warbler‡</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Dendroica fusca</td>
<td>Blackburnian warbler‡</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dendroica castanea</td>
<td>Bay-breasted warbler</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Setophaga ruticilla</td>
<td>American redstart‡</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Piranga olivacea</td>
<td>Scarlet tanager†</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Seiurus aurocapillus</td>
<td>Ovenbird†</td>
<td>7</td>
<td>12</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Icterus galbula</td>
<td>Northern oriole‡§</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pheucticus ludovicianus</td>
<td>Rose-breasted grosbeak</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sitta carolinensis</td>
<td>White-breasted nuthatch</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bombycilla cedrorum</td>
<td>Cedar waxwing</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cyanocitta cristata</td>
<td>Blue jay†‡§</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>28</td>
<td>39</td>
<td>40</td>
<td>43</td>
</tr>
</tbody>
</table>

*Species that are known to consume gypsy moth larvae: †Smith & Lautenschlager (1981); ‡JSE (unpublished data); §Forbush & Fernald (1896); ¶Whelan, Holmes, & Smith (1989).

---

Fig. 4. Percentage mortality due to parasitoids during period 2 and period 3 observed by rearing gypsy moth larvae collected from plots at four initial densities: Compsilura concinnata (■); Phobocampe disparis (■); Cotesia melanoscela (■); Parasetigena silvestris (■).
attacks by *C. concinnata*. The only other mortality agent for which the regression was significant was *P. disparis*, but for this parasitoid the slope of the regression line was negative (Fig. 5c).

At the beginning of period 3 when gypsy moths were in the late larval instars there was substantial parasitism (Fig. 4). Mortality due to *C. concinnata* was density-dependent (Fig. 6a) although the overall mortality for this period (*k₃*) did not show this trend (Fig. 3c). Again the response of *C. concinnata* was non-linear with a quadratic model (*Y=25·16 – 13·60 X + 1·84 X²*) providing a significantly better fit than the linear model (*P=0·012, F=14·95, d.f. = 1,5*). At this range of densities the density-dependent response levelled off at the lower densities (Fig. 6a). None of the regressions for the other mortality agents acting during this period was significant.

The density dependence of oviposition by *P. silvestris* reversed over time. On the first two sampling occasions the relationship between attack rate and density was inversely density-dependent (Fig. 7a, b). The third sample showed no significant relationship (Fig. 7c) yet for the fourth sample there was a strong positive density-dependent relationship (Fig. 7d).

Additional evidence of density-dependent mortality during the mid and late larval instars was obtained from the transect line data. The total number of larvae under bands...
declined with distance from the plot, as we would expect from the limited dispersal of first and late instar larvae (Mason & McManus 1981). The proportion of dead larvae also decreased significantly with distance from the plot (\( P = 0.020, F = 7.37, \text{d.f.} = 1,11 \)) indicating that mortality was higher closer to the release plots where densities were higher.

The rate of mortality during period 4 was higher than during any other period in all but one plot (Table 3), but there was no evidence of an overall density-dependent reduction in pupal density. The pupal deployment experiment suggested that predation on pupae was inversely density-dependent (Table 5). The survival of both male and female pupae was greatest in Plots 1B and 2B, which were the two plots with the most gypsy moth larvae m\(^{-1}\) of burlap band prior to the experiment. These two plots were also two of the three plots in which egg masses were found. Survival of deployed female pupae in plot 2A was not significantly greater than in the other plots, but survival of females under burlap bands was 100%. The major predators were probably small mammals because male pupae survived well inside wire cages. The carabid beetle *Calosoma sycophanta* L. is also too large to go through the mesh of the wire cages. It has been implicated as an important predator in high density populations (Weseloh 1985) but we saw little evidence of it under burlap bands (where it is often found) in any of our plots. None of the surviving pupae that we collected and reared yielded pupal parasitoids such as *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae), and there were no *B. intermedia* emergence holes in any of the pupal cadavers collected under burlap bands.
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Fig. 7. Regressions of the $k$-values of the oviposition rate of *P. silvestris* on four weekly sampling occasions on $\log_{10} N_t$ (the density of larvae prior to the sampling occasion).

Table 5. Survival of male (M) and female (F) pupae deployed in the litter, survival of male pupae in exclosures (MX), density and survival of pupae under burlap bands, and number of egg masses ha$^{-1}$ at the end of the season.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Proportion surviving 3 days*</th>
<th>Number m$^{-1}$ burlap</th>
<th>Proportion surviving</th>
<th>Number of egg masses ha$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>MX</td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>0.48bc</td>
<td>0.38bcd</td>
<td>0.98a</td>
<td>0.08</td>
</tr>
<tr>
<td>1B</td>
<td>0.79a</td>
<td>0.62a</td>
<td>0.98a</td>
<td>0.42</td>
</tr>
<tr>
<td>2A</td>
<td>0.50c</td>
<td>0.36ce</td>
<td>0.92a</td>
<td>0.04</td>
</tr>
<tr>
<td>2B</td>
<td>0.77a</td>
<td>0.59ab</td>
<td>0.98a</td>
<td>0.70</td>
</tr>
<tr>
<td>3A</td>
<td>0.52bc</td>
<td>0.34ce</td>
<td>0.98a</td>
<td>0.01</td>
</tr>
<tr>
<td>3B</td>
<td>0.49bcd</td>
<td>0.50ac</td>
<td>1.00a</td>
<td>0.45</td>
</tr>
<tr>
<td>4A</td>
<td>0.40bc</td>
<td>0.27de</td>
<td>0.90a</td>
<td>0.14</td>
</tr>
<tr>
<td>4B</td>
<td>0.28de</td>
<td>0.34ce</td>
<td>1.00a</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Means followed by the same lower case letter were not significantly different at $P<0.05$, Lee-Desu $D$ statistic (Lee & Desu 1972).
DISCUSSION

Contrary to the suggestion of many investigators, we have evidence that parasitoids have an important impact on the population dynamics of the gypsy moth and can, in combination with predation, cause more than 99% mortality within a generation. We found no NPV, a common cause of mortality in high density populations (Campbell 1967; Doane 1970b), yet populations in all eight plots declined in a density-dependent fashion to quite low levels. Predators caused a high rate of mortality during the pupal stage but not in a positively density-dependent manner. Rather, the density-dependent mortality occurred during the early and mid instars.

Similar responses of gypsy moth parasitoids have been noted by S. Wilmot et al. (personal communication) for releases of F1 sterile larvae (progeny of partially sterilized adults) in Vermont and by Liebhold & Elkinton (1989) for releases of both F1 sterile and feral larvae on Cape Cod, Massachusetts. Also, a number of previous research projects (M. L. McManus, personal communication) have tried yet failed to create high density populations of gypsy moth larvae by releasing egg masses, indicating a strong response by natural enemies. These results suggest that the strong response of parasitoids to local increases in host density is common and consistent in many places in different years.

The drop in density from hatch to third instar was positively density-dependent, but we were unable to determine its cause. Parasitoids had not begun to appear in samples, and we found no evidence of resident birds aggregating in areas of high gypsy moth density. However, migrant birds may have removed a higher proportion of larvae in the higher density plots. Furthermore, the resident birds may have foraged more intensively for gypsy moth larvae in the high density plots (Furuta 1976).

Dispersal of first instars may also be density dependent (Campbell 1969; Semevsky 1971). Leonard (1970) suggested that the lower nutrition of eggs laid in high density populations resulted in an increased probability of dispersal, but this could not account for our results because all the eggs for this experiment were collected from the same population.

The polyphagous parasitoid, C. concinnata, was the major mortality agent in our plots during the mid larval stage, and it acted in a positively density-dependent fashion. This parasitoid showed a remarkable ability to locate and parasitize gypsy moths in our experimental populations. Gypsy moths were not present in the plots prior to our release, yet we measured up to 55% mortality due to this parasitoid in a single week. Beddington, Free & Lawton (1978) suggested that successful natural enemies are most probably specialists, but in our study the more specialized parasitoids, such as P. disparis and C. melanoseca, did not respond as strongly as C. concinnata to increases in pest densities. Polyphagous natural enemies can remain abundant when a particular host species has become extremely sparse or locally extinct, and when reinestation of the host occurs they can respond quickly (Murdoch, Chesson & Chesson 1985). This seems to be what occurred in our study. C. concinnata was present in the area and was able to respond by either migrating from surrounding areas or switching to gypsy moths from alternative hosts within the plots.

Compsilura concinnata has up to four generations per year (Culver 1919), and requires hosts other than the gypsy moth during some of these generations. The availability of alternative hosts, therefore, may be an important factor in determining parasitoid abundance from one year to the next. In an inundative release of C. concinnata in
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Pennsylvania, Blumenthal, Fusco & Reardon (1979) found significantly higher percentage parasitism in release plots than in control plots in the year of release, but not the following year.

Another factor that might limit the effectiveness of this parasitoid in regulating gypsy moth populations is the spatial scale on which gypsy moth populations typically increase in density. We recorded a density-dependent response to hosts in areas of 1 ha. An increase in density over larger areas might overwhelm the pool of available parasitoids and populations could continue to increase. There is preliminary evidence, however, that C. concinnata can respond to population increase over larger areas (T. M. ODell, personal communication). In plots of 16.7 and 57.5 ha in Vermont, F1 sterile gypsy moth larvae were released at rates of 1 million and 600,000 larvae hatching ha\(^{-1}\), respectively. At the end of the season both plots contained only one egg mass ha\(^{-1}\). A major mortality agent in these plots was C. concinnata.

The possible non-linearity of the density-dependent response of C. concinnata is also an important consideration. A positive density-dependent response may occur only when there are approximately 6000–100,000 larvae ha\(^{-1}\) (Figs 5a, 6a). Outside this range, the response may be inversely density-dependent or density-independent. The ability of C. concinnata to regulate populations at extremely low or high densities is, therefore, uncertain.

The response of the oligophagous parasitoid, P. silvestris, was initially inversely density-dependent but became positive as the season progressed. One plausible explanation for this pattern would be that more P. silvestris females were attracted to the high density plots, but at the beginning of the season there were so many larvae in these plots that inversely density-dependent parasitism nevertheless occurred. As host densities declined, due primarily to the action of C. concinnata, the greater number of P. silvestris females in the higher density plots attacked a greater proportion of larvae. Alternatively, there may be greater continued recruitment of P. silvestris females to the high density plots.

It should be noted that the density-dependent oviposition by P. silvestris that occurred during the week of 3 July was not evident when we examined mortality over the entire period 3 (3–16 July). One possible explanation is that the early inverse density-dependent attack rate cancelled the later direct density-dependent attack rate so that the overall effect was not density-dependent. Another explanation is that most mortality due to P. silvestris occurred during period 4, when population densities were too low to sample for estimates of mortality. This is possible because P. silvestris emerges just prior to pupation, which in our plots was occurring during period 4.

Whether spatially density-dependent responses can lead to temporal density dependence and population regulation has recently been debated. In simulations (e.g. Beddington, Free & Lawton 1978; Hassell 1985) a spatially density-dependent response among subpopulations can contribute to long-term stability of populations. In these models, the number of parasitoids in a particular generation depended on the number of hosts parasitized in the previous generation, as might be expected for P. silvestris. The stabilizing ability of a generalist natural enemy such as C. concinnata, which has a strong spatially density-dependent response but, presumably, little generational carryover, is less clear. The model of Hassell (1985) has predicted that spatially density-dependent mortality by such agents can stabilize a population, but this occurs only if the degree of clumping of the host within subpopulations changes as overall density changes (Latto & Hassell 1988). We would expect such a change in the dispersion pattern of gypsy moths
following the action of density-dependent natural enemies because females do not fly and dispersal of first instars is limited (Mason & McManus 1981).

Life-table analyses of gypsy moth population dynamics in North America have so far failed to identify regulation or temporal density-dependent mortality due to parasitoids (Dempster 1983). There is some evidence of density-dependent pupal mortality, presumably due to predation (Campbell, Hubbard & Sloan 1975; Campbell & Sloan 1978), but spatial and temporal density-dependent effects were considered together in these studies and there were few or no data on the contribution of parasitoids to overall mortality. It may be that populations are not regulated around an equilibrium density, but are instead characterized by local extinction and colonization which results in area-wide stability (e.g. Murdoch, Chesson & Chesson 1985; Nachman 1987; Morrison & Barbosa 1987; Elkinton et al. 1989). Persistence of such populations is most likely when there is some density-dependent coupling of hosts and parasitoids, and when migration rates of the parasitoid exceed those of the host (Reeve 1988). This would be possible for gypsy moth populations because gypsy moths do not generally migrate far and parasitoids can migrate further than their hosts and can respond in a density-dependent fashion.

Another reason why regulation and temporal density dependence may not have been detected is that few long-term studies have been conducted. Even in long-term studies, however, stochastic variation (Hassell 1985, 1987) or the use of an inappropriate spatial scale (Heads & Lawton 1983; Hassell, Southwood & Reader 1987) may obscure density-dependent processes. Furthermore, host density or parasitism is difficult to measure precisely when gypsy moth densities are low, therefore such populations are rarely studied. If regulation is the result of rapid suppression of local increases in gypsy moth density, it is unlikely that previous studies would have detected these processes.

Further experimental manipulations of low density gypsy moth populations are needed to resolve the question of population regulation. If such populations are indeed regulated, parasitoids attacking the mid instars, not pupal predators, may be the agents responsible. Campbell & Sloan (1978) theorized that gypsy moth populations are characterized by a low density threshold above which mortality by small mammal predators is inversely density-dependent and populations escape into outbreak phase (Elkinton et al. 1989). During the periods of low population density between outbreaks, parasitoids such as C. concinnata and P. silvestris may suppress local increases in the density of gypsy moths to levels at which predation by small mammals would cause sufficient mortality to prevent population increase. Because C. concinnata is multivoltine and is dependent on the availability of alternative hosts, however, this parasitoid may not be present in large numbers in certain years. Lowered mortality from C. concinnata in a given year might result in more pupae than could be consumed by pupal predators, leading to an increase in reproduction by the gypsy moth. The number of host larvae the following year might then be outside the range over which the density-dependent response of C. concinnata occurs. These processes could rapidly propel the population above the thresholds of both parasitoids and predators and into an outbreak phase.

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Density dependence in gypsy moth populations

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Density dependence in gypsy moth populations


(Received 30 September 1988)
We calculated the marginal probability of mortality due to a mortality agent in the presence of other simultaneous mortality agents (after Royama 1981b). The marginal probability can be thought of as the proportion that would have died from a given agent if none of the other agents was present. For a system with two simultaneous parasitoids (A and B) the marginal probabilities ($m_A, m_B$) are:

\[
\begin{align*}
    m_A &= \frac{v_A}{1 - cm_B} \\
    m_B &= \frac{(c-1)v_A + cv_B + 1 - [(v_A - cv_A - cv_B - 1)^2 - 4cv_B]^{1/2}}{2c}
\end{align*}
\]

where $v_A =$ proportion of reared hosts producing parasitoid A; $v_B =$ proportion of reared hosts producing parasitoid B; $c =$ proportion of hosts that yield parasitoid B when both A and B attack the same individual; $1 - c =$ proportion of hosts that yield parasitoid A when both A and B attack the same individual. These formulae assume that either parasitoid A or B, but not both, can emerge from hosts parasitized by both species. In our calculations we assumed $c = 0.5$ for all interactions. This value is largely unknown for interactions between gypsy moth parasitoids but for the interaction between C. concinna and C. melanoscela, the parasitoids emerged in equal proportions from multiparasitized host larvae (Weseloh 1983).

We extended this analysis to four simultaneous parasitoids and one disease (unexplained rearing mortality) by analysing each agent separately against all other agents combined. In other words we defined $v_B$ as death due to all four of the other simultaneous agents. This scheme caused a small error in partitioning the marginal probabilities for host individuals attacked by three or more agents simultaneously, but this constituted an extremely small fraction of the mortality and the resulting error in the estimated $k$-values was less than 1%.