Performance of Wild and Laboratory-Reared Gypsy Moth (Lepidoptera: Erebidae): A Comparison between Foliage and Artificial Diet

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ABSTRACT The effects of long-term mass rearing of laboratory insects on ecologically relevant traits is an important consideration when applying research conclusions to wild populations or developing management strategies. Laboratory strains of the gypsy moth, *Lymantria dispar* (L.), an invasive forest pest in North America, have been continuously reared since 1967. Selection on these strains has enhanced a variety of traits, resulting in faster development, shorter diapause, and greater fecundity. As in many mass-reared insects, laboratory strains of the gypsy moth are also reared exclusively on artificial diets that lack much of the phytochemical and nutritional complexity associated with natural foliage. We tested for differences in growth and development of wild gypsy moth populations from across the invasive range in comparison to laboratory strains when reared on artificial diet and a preferred foliage host species, northern red oak (*Quercus rubra L.*). Overall, caterpillars reared on foliage had higher survival and faster development rates, with smaller differences among populations. When reared on artificial diet, laboratory strains had the highest performance as expected. The response from the wild populations was mixed, with two populations performing poorly on artificial diet and another performing nearly as well as the laboratory strains. Performance on diet was enhanced when larvae received cubed portions changed regularly, as opposed to filled cups. Understanding these relationships between food source and population performance is important for informing studies that examine population comparisons using wild and laboratory-reared strains.

KEY WORDS adaptation, artificial diet, gypsy moth, population variation, rearing

Development of mass-rearing techniques and artificial diets have enabled important research on the ecology and biology of insect pests (King and Leppla 1984, Sørensen et al. 2012). Optimized rearing protocols have been developed for a wide variety of species and much is known about the benefits and drawbacks of mass rearing (Singh and Moore 1985, Smith 2012). The availability of insects and high production volume from mass-rearing facilitates a wide variety of research efforts that would otherwise be infeasible, particularly in the field of biological methods for integrated pest management. Large-scale testing of management actions such as trapping efficacy or treatment programs often requires large numbers of insects, as does sterile releases to overwhelm a wild population (Parker 2005).

Mass rearing for insect production can result in well-known consequences to colony quality, such as reductions in genetic variability and artificial selection that can affect a wide array of phenotypic traits (King and Leppla 1984). Mass production often focuses on increasing output and deliberate or inadvertent selection can result in traits that optimize rapid growth, shorter development, and reduced or even loss of diapause under artificial conditions. Such selection can alter performance relative to wild type individuals in natural environments. For example, experimental populations of *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae) mass reared for biological control had greater fecundity, lower survival, and less sensitivity to diapause-inducing cues than wild populations (Hough-Goldstein et al. 2014). Consideration of these effects can be important when translating results from laboratory-reared strains to conclusions about wild populations.

An exemplar mass-rearing program was developed by the U.S. Department of Agriculture (USDA) for production of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), for research and management. A laboratory colony was started from a single wild population in 1967, and the New Jersey Standard Strain (NJSS) has been in continuous cultivation since then without outcrossing (Keena et al. 1998). A primary

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focus of the early program was increasing output to facilitate the development of the gypsy moth nucleopolyhedrosis virus as a biological control technique. Thus, colony establishment and maintenance efforts were focused on reducing larval maturation times and enhancing fecundity to increase virus production (Keena and Odell 1994). Since establishment, this laboratory strain has been used in countless studies of gypsy moth ecology, flight, behavior, and physiology (e.g., Elkinton and Carde 1980, Gould et al. 1990, Werren et al. 1992, Lindroth et al. 1997, Barbehenn et al. 2014, to name but a few).

The gypsy moth is also a model system for studies of invasion biology because of the detailed knowledge of its biology and its well-documented introduction and spread in Eastern North America (Tobin et al. 2012). Accidentally released in Medford, MA, in 1869, the concentric spread of the gypsy moth is known in detail from county quarantine records, aerial defoliation surveys, and pheromone-baited traps along the gypsy moth invasion front (Liebhold et al. 1992, Sharov et al. 2002). The gypsy moth has long been viewed as a wide-ranging generalist where phenotypic plasticity has facilitated establishment across a wide variety of environments. However, the potential for evolutionary changes and local adaption in introduced species has been demonstrated as an important component of invasions in a wide variety of taxa (Mooney and Cleland 2001, Sakai et al. 2001). Considering the climatically diverse and environmentally distinct regions invaded by the gypsy moth (currently ranging from Southern Canada and Minnesota to North Carolina) and its gradual spread across the landscape over the past century, divergence and local adaptation seem likely despite the putative bottleneck experienced at its introduction (Bogdanowicz et al. 1997). Whether important traits in the gypsy moth have changed across the environmental gradient of the current range and how shifts in life history could impact further spread is an ecologically and economically important question.

In addition to laboratory selection for altered developmental traits, artificial diets used in high-output facilities may impose selective regimes (Vanderzant 1974). The standard diet for gypsy moth is a high wheat germ formulation (Bell et al. 1981). Continuous use of these novel diets can select for genotypes that maximize performance on a substrate very different than natural foliage. Gypsy moth larvae are generalist folivores, feeding on >300 host trees (Liebhold et al. 1995). Although many studies have compared the feeding and growth responses among different tree species (e.g., Hough and Pimentel 1978, Raupp et al. 1988, Roden and Surgeoner 1991, Stoyenoff et al. 1994), fewer have quantified relative performance on foliage and artificial diet (e.g., Barbosa et al. 1983, Hajek 1989). One of the limits to ecological studies using wild populations is the effort and logistics of rearing on foliage; thus, research is needed to determine the growth responses of wild populations on artificial diet and the potential to use advancements from mass rearing to facilitate ecological studies. Previous studies comparing laboratory and wild populations have generally used a single population from the core of the established range (Doane and McManus 1981).

In this study, we conducted three field and laboratory experiments to compare the relative survival, growth, and development of several wild gypsy moth populations and two NJSS laboratory populations when reared on either standard commercial artificial diet or northern red oak (*Quercus rubra* L.) foliage. We compared wild populations from the established range as well as populations from the northern and southern range limits. We tested the expectation that the selective breeding in the laboratory strain has resulted in faster development and higher growth rates compared with wild populations, and that wild populations from across the invasive range would have equivalent performance under common rearing conditions. We also compared performance based on natural versus artificial diet and tested the hypothesis that wild populations would have reduced performance on artificial diet relative to the NJSS and, conversely, that diet-adapted laboratory strains would have reduced performance on foliage relative to wild populations.

Materials and Methods

Study Populations. We established populations of gypsy moth from across the latitudinal range of the invasion in North America by collecting egg masses from Quebec City, Quebec, Canada (QC), Milford, MA (MASS), and Manassas, Virginia (VA). All collected egg masses were surface sterilized with 10% formalin before rearing. The MASS population is located 72 km from the original introduction site of the gypsy moth invasion. To protect against confounding maternal effects from population density or host quality (Rossiter 1991), we reared each population through at least one generation on northern red oak foliage in an outdoor array under ambient temperatures at Lafayette Road Field Station in Syracuse, NY. Egg masses from 20 to 40 females were broken apart and mixed together to homogenize genetic diversity, and 100–150 neonates were drawn from this pool at hatching. First and second instars were initially housed in large plastic petri dishes and transferred at third instar to 18.9-liter plastic buckets covered in spun polyester mesh (commercially available as crop row covers). Larvae were exclusively fed fresh cut northern red oak foliage every 3–4 d until pupation, with all larvae receiving foliage from the same tree on the same day. Oak branches were cut under water and inserted into plastic containers with water to maintain turgor (floral water tubes in the smaller containers and flasks in the larger buckets). Emerged adults were placed in paper-lined buckets for mating and oviposition of eggs for the next generation. Gypsy moth is univoltine and eggs were overwintered under outdoor conditions near Syracuse, NY. The eggs used in our experiment were from the F_2 generation for MASS and F_3 generation for QC and VA. In addition to these three populations, we also used a population collected in 2014 from near Blairstown, New Jersey (NJ) at the location historically used to establish the NJSS. To minimize maternal effects in the first

generation of this population, eggs were collected solely from northern red oak trees in a low-density population.

Laboratory-reared NJSS egg masses were obtained from two sources: the Animal and Plant Health Inspection Service (APHIS), Center for Plant Health Science and Technology, Buzzards Bay, Massachusetts, Buzzards Bay, MA (substrain NJSS-APHIS), and the Forest Service's Northern Research Station Laboratory in Hamden, CT (substrain NJSS-FS). The NJSS was initially founded by APHIS in 1967 and the NJSS-FS substrain was established in 1980 using NJSS-APHIS egg masses (Keena and Odell 1994). Since establishment, each substrain has been reared continuously in isolation at each site and these two strains have developed some trait and performance differences (Keena et al. 1995). In addition to obtaining NJSS reared on artificial diet directly from the laboratories shortly after oviposition in 2014, we previously obtained NJSS-APHIS eggs in 2011 and reared them on foliage in the standardized array, as described above for two consecutive generations. The foliage-reared laboratory strain used in our experiment was the F_3 generation for this group (denoted as NJSS-A-Foliage).

We used these populations in three experiments conducted during the spring and summer of 2014: an experiment rearing larvae under outdoor conditions in New York on foliage, a growth chamber experiment under a constant rearing temperature on artificial diet, and a growth chamber experiment under a cycling temperature regime using both food sources (hereafter referred to as "diet" and "foliage"). The first two experiments were conducted in Syracuse, NY, and the third experiment in Richmond, VA. All eggs used in these experiments were initially overwintered outdoors in Syracuse. Batches of eggs for the third experiment were transferred to Richmond in January 2014 and completed diapause at 4–8°C until just before the onset of spring. Eggs used in experiments in Syracuse were placed in cold storage just prior to the onset of spring. For all experiments, we synchronized egg hatch among populations with local oak bud burst (mid-April for Richmond and mid-May for Syracuse). We achieved synchronous hatching by allowing a subset of eggs to hatch at room temperature to determine the number of days until emergence for each population and then removed eggs from cold storage accordingly. After the onset of hatching, egg masses in all experiments were allowed to cumulatively hatch for 2 d before selecting larvae to avoid biases due to the timing of hatch. Very little overwintering egg mortality was observed in any of our experimental populations.

New York Foliage Experiment (Syracuse, NY). We compared gypsy moth development on foliage under ambient outdoor New York temperatures across six populations (QC, MASS, NJ, NJSS-APHIS, NJSS-A-Foliage, NJSS-FS). Hatching was timed to coincide with bud break and 30–40 larvae were haphazardly selected and placed in four replicate 1-liter Tupperware containers for each population on 12 May 2014. Northern red oak foliage was provided as described above, and replaced every 4 d for the duration of larval development. When larvae reached third instar, they were transferred to mesh screened 18.9-liter plastic buckets to accommodate the larger volumes of foliage required. Larvae were reared in a screened and shaded outdoor insectary at field temperatures. Fully sclerotized pupae were removed, weighed, and placed individually in 44-ml plastic cups and sealed with a snap cap. Cups were returned to the insectary and checked daily for emergence and scoring of adult sex. Mean pupal mass and developmental time to adult male and female emergence was calculated for each replicate enclosure.

Constant Temperature Diet Experiment (Syracuse, NY). We compared gypsy moth growth and development on diet in a growth chamber experiment at a constant 28°C across five populations (MASS, NJ, NJSS-APHIS, NJSS-A-Foliage, and NJSS-FS). Limits on growth chamber capacity prevented inclusion of the QC population in this experiment. Three replicates of 30 larvae from each population were placed in 180-ml waxed paper cups on 4 June 2014. Each cup held artificial diet (USDA, Hamden Formula Gypsy Moth Diet #F9630B, Bio-Serv, Flemington, NJ) poured to an approximate depth of 1 cm before solidifying. Cups were housed in a single Percival environmental chamber at a photoperiod of 14:10 (L:D) h. We chose 28°C as the rearing temperature for this experiment because it results in the fastest rates of larval development (Logan et al. 1991). Larvae were transferred to new cups with fresh diet at 5-d intervals to avoid mortality due to diet drying. After 2 wk, the caterpillars in each cup were weighed and redistributed using an additional cup to reduce the density in each cup. At this point, based on the initial results of the third experiment, diet was provided as cubes for the remainder of development. Pupae were handled as above and remained in the growth chamber until emergence. In addition to calculating mean pupal mass and developmental time as above, we also examined larval growth over the first 2 wk of development in this experiment. Larvae from each cup were group weighed and we divided the total mass in each cup by the number of individuals to obtain the mean larval mass per replicate enclosure.

Foliage and Diet Comparison under Diurnal Cycles (Richmond, VA). We conducted a growth chamber experiment to directly compare growth and development across five populations (NJSS-APHIS, NJSS-A-Foliage, QC, MASS, and VA) on three diet treatments: northern red oak foliage, artificial diet provided in cubed portions and changed regularly (denoted as the diet cube treatment), and larvae from the diet cube treatment switched onto artificial diet provided in filled cups for the second half of development (denoted as the diet-filled treatment). The diet-filled treatment was added as a comparison to mass-rearing protocols for gypsy moth, where large portions of diet are provided in a single cup for the majority of development (Singh and Moore 1985). The combined batches of eggs for each population were allowed to cumulatively hatch for 2 d and then 60 larvae were randomly selected for the foliage treatment and the diet

cube treatment. In each treatment, larvae were initially reared in groups of 10, with six replicates per population and diet treatment. The experiment began on 18 April 2014, timed to coincide with the bud burst of northern red oak.

Larvae in the foliage treatment were housed in 1-liter paper cups with a plastic lid to accommodate a small cluster of leaves placed in a floral water tube. Northern red oak foliage from a local source (Hollywood Cemetery, Richmond, VA) was provided every 3–4 d. Larvae in the diet cube treatment were housed in 180-ml plastic cups with a paper lid and provided with a 4 cm^2 of gypsy moth diet every 3–4 d (same formulation as above). Larvae were transferred to fresh cups at each diet change. Rearing containers were housed in two environmental chambers, with each treatment split evenly between the two chambers. Both chambers had a photoperiod of 14:10 (L:D) h and temperature fluctuated on a daily cycle between 12°C at night and 24°C during the day, the average minimum and maximum temperatures for Richmond, VA, over the past 10 yr for mid-April to mid-May. This temperature cycle was chosen to maintain caterpillar development rates in parallel with local foliage maturation. Relative humidity was maintained between 60 and 80%.

Larvae were separated for rearing in individual containers on 9 May 2014, 3 wk after the initiation of the experiment. Five larvae from each of the six cups were selected for a total of 30 replicates of each treatment per population. We visually selected the largest, smallest, and three mid-size larvae from each cup to maintain within treatment variation and avoid unintentional biases. Larvae in the foliage treatment were maintained in the 1-liter cups as before, but with one caterpillar per cup and a reduced portion of foliage. Larvae in the diet cube treatment were housed individually in 60-ml plastic cups and provided a 2 cm^2 of artificial diet. For four populations (NJSS-APHIS, QC, MASS, and VA), we took the remaining larvae from the diet cube treatment and housed them in 75-ml plastic cups half filled with artificial diet that was not changed for the remainder of the experiment (the diet-filled treatment). This treatment was designed to represent the gypsy moth mass-rearing protocol, where the artificial diet solidifies directly in the rearing cup and gypsy moth larvae feed on this medium for 5–6 wk until the completion of development (Singh and Moore 1985). The sample sizes for the diet-filled treatment were 24 for QC, 22 for MASS, 24 for VA, and 21 for NJSS-APHIS (as not all 60 larvae survived the first stage of the experiment and the 30 larvae continuing in the diet cube treatment were allocated before assigning larvae to the filled treatment).

Individual larvae were monitored for survival and development at diet changes and weighed after 2 wk on 23 May to measure growth. After reaching fifth instar, larvae were monitored daily for pupal formation and emergence. As in the previous two experiments, we examined sex-specific pupal mass and developmental time as dependent variables. We compared growth between populations and diet treatments during the two stages of the experiment. For growth during the first stage of the experiment when caterpillars were group reared in two treatments (foliage and diet cube), we compared mass measurements recorded on 9 May. We averaged the mass of caterpillars from the same cup to obtain the mean larval mass per replicate enclosure. For the second stage of the experiment, we calculated individual relative growth rate as $[\ln(m_2) - \ln(m_1)]/t$, using the 9 May mass (m_1) and mass recorded again 2 wk later (m_2), where t is time in days.

Analysis. In the New York foliage experiment and the optimal temperature diet experiment, we analyzed differences in pupal mass and developmental time between populations using individual general linear models, with separate analyses for males and females owing to differences between the sexes in number of instars. For each analysis, we conducted an *a priori* planned comparison between wild populations and laboratory strains. We also used *post hoc* Tukey tests to compare the differences between all populations within each analysis. In the diet experiment, we used a general linear mixed model to test for population differences in mass after 2 wk of growth, with the number of larvae per rearing container included as a random effect. For this analysis, we also conducted an *a priori* comparison between wild populations and laboratory strains.

In the growth chamber experiment comparing foliage and diet using a cycling temperature regime, we used logistic regression to examine differences in survival between the main effects of population and diet. We examined the effect of the diet treatments, populations, and their interaction on log-transformed mass after stage 1, using a two-way general linear mixed model, with chamber assignment included as a random effect. We used the same model to test the effect of diet treatment and population on relative growth rate in the second stage of the experiment and sex-specific pupal mass and developmental times. Given low survivorship to pupation in the filled cup treatment (see Results), we restricted statistical analyses to the foliage and diet cube treatment. Dependent variables in the linear models were natural log-transformed to achieve normality and statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NC).

Results

Development on foliage in the New York experiment was relatively consistent across populations, with no significant differences in pupal mass among populations for females or males (Table 1). For both analyses with males and females, the comparison between wild and laboratory strains in pupal mass was also not significant ($F < 1.84$; df = 1, 23; $P > 0.19$). There were significant differences between populations in developmental time on foliage (Table 1) and the contrast between wild and laboratory strains was significant for males ($F = 6.98$; df = 1, 23; $P = 0.02$) but not females ($F = 0.38$; df = 1, 23; $P = 0.55$). These differences in development time were small, with the slowest population (QC) averaging 2.1 and 1.1 d longer in total developmental time for

Table 1. Pupal mass and developmental time (Dev. time) for female and male gypsy moth from the first two experiments reported as mean (± 1 SE)

	Population						*F*	*P*-value
	QC	MASS	NJ	NJSS-A-F	NJSS-FS	NJSS- APHIS		
Females								
Foliage (outdoors)								
Dev. time (d)	59.0 (0.69)^{a}	57.6 (0.24)^{ab}	57.1 (0.28)^{b}	58.7 (0.29)^{ab}	57.6 (0.43)^{ab}	57.0 (0.41)^{b}	4.05	**0.01**
Pupal mass (g)	0.98 (0.08)^{a}	0.88 (0.01)^{a}	0.97 (0.05)^{a}	0.92 (0.05)^{a}	0.87 (0.05)^{a}	0.99 (0.04)^{a}	1.05	0.42
N	54	51	46	64	33	33		
Artificial diet (28°C)								
Dev. time (d)	—	52.4 (1.10)^{a}	51.9 (0.30)^{a}	48.5 (3.15)^{ab}	44.0 (2.27)^{b}	45.8 (1.95)^{ab}	4.67	**0.009**
Pupal mass (g)	—	1.00 (0.09)^{a}	1.06 (0.09)^{ab}	1.39 (0.10)^{ab}	1.34 (0.05)^{ab}	1.41 (0.11)^{b}	4.48	**0.001**
N	—	20	25	11	11	10		
Males								
Foliage (outdoors)								
Dev. time (d)	56.9 (0.22)^{a}	56.4 (0.14)^{ab}	56.7 (0.18)^{a}	56.6 (0.09)^{ab}	56.5 (0.22)^{ab}	55.8 (0.31)^{b}	3.78	**0.02**
Pupal mass (g)	0.43 (0.01)^{a}	0.41 (0.01)^{a}	0.43 (0.01)^{a}	0.42 (0.01)^{a}	0.43 (0.01)^{a}	0.44 (0.01)^{a}	1.32	0.30
N	54	66	50	55	44	58		
Artificial diet (28°C)								
Dev. time (d)	–	47.8 (1.49)^{a}	49.0 (1.10)^{a}	39.8 (2.70)^{b}	33.2 (0.59)^{c}	33.5 (0.74)^{c}	29.45	**<0.001**
Pupal mass (g)	–	0.38 (0.02)^{ab}	0.36 (0.02)^{a}	0.46 (0.02)^{b}	0.46 (0.01)^{b}	0.46 (0.02)^{b}	5.90	**0.002**
N	–	16	27	14	12	17		

Sample sizes (*N*) of males and females is reported for each experiment. Foliage indicates the experiment rearing gypsy moth populations on foliage under outdoor conditions. In the artificial diet experiment, gypsy moths were reared in a growth chamber at a constant temperature regime of 28°C. *F* and *P* are statistics derived from separate ANOVAs testing the overall effect of population on each variable (df = 5, 23 for foliage comparisons and df = 4, 22 for artificial diet comparisons). Bolded values indicate significance in the overall model at $P < 0.05$. Means within the same row with the same superscript letter are not significantly different according to *post hoc* Tukey comparisons. Populations in these experiments included wild populations (QC, Quebec City, Quebec, Canada; MASS, Milford, Massachusetts; and NJ, Blairstown, New Jersey) and laboratory strains (NJSS-APHIS, New Jersey Standard Strain reared at an APHIS Laboratory; NJSS-FS, New Jersey Standard Strain reared at a Forest Service Research Center; and NJSS-A-Foliage, the APHIS strain reared on foliage for three generations).

females and males, respectively, compared with the fastest developing population (NJSS-APHIS).

We found larger differences between populations when larvae were reared on artificial diet in the growth chamber experiment at a constant 28°C. The effect of population was significant in all tests of pupal mass and developmental time for males and females (Table 1). Comparisons between wild and laboratory strains revealed highly significant differences for both sexes in pupal mass ($F > 17.66$; df = 1, 22; $P < 0.0005$) and development time ($F > 15.56$; df = 1, 22; $P < 0.001$), with wild populations having lower pupal mass and slower developmental rates. Differences in developmental time were pronounced on artificial diet, with the slowest populations (MASS and NJ) averaging 8.4 and 15.8 d longer in total developmental time for females and males, respectively, compared with the fastest developing population (NJSS-FS). Growth on artificial diet during the early stages of development was also much more rapid in the laboratory strains, with significantly higher mass at 12 d posthatching compared with wild populations ($F = 1469.89$; df = 1, 22; $P < 0.0001$; Fig. 1). Sample sizes of larvae surviving to pupation were lower in the diet experiment because of microbial contamination, which removed individuals from the experiment (Table 1).

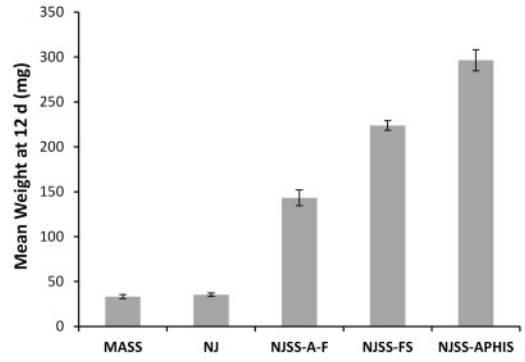

Fig. 1. Larval growth during the first 12 d of development when reared on artificial diet in a growth chamber at a constant temperature regime of 28°C. Populations in this experiment included wild populations (MASS, Milford, Massachusetts; and NJ, Blairstown, New Jersey) and laboratory strains (NJSS-APHIS, New Jersey Standard Strain reared at an APHIS Laboratory; NJSS-FS, New Jersey Standard Strain reared at a Forest Service Research Center, and NJSS-A-Foliage, the APHIS strain reared on foliage for three generations).

In the growth chamber experiment that compared foliage and diet under a cycling temperature regime, survival during the first stage of the experiment with group rearing was high. During this 3-wk period, 84% of larvae in the foliage treatment and 88% of larvae in the diet cube treatment survived. The majority of larvae in the foliage experiment were fourth instars at the start of individual rearing (averaging 90% at the fourth-instar stage across populations) and larvae in the diet cube treatment were mostly third instars (averaging 85% at the third-instar stage across populations). During the second stage of the experiment, after being split into three diet treatments, survival was highest on foliage across all populations, with reductions in survival on diet cubes for the wild populations (Table 2).

Table 2. Percentage of larvae surviving to pupation during the second stage of the foliage and diet comparison experiment, where larvae were reared and monitored individually

Population	Food source		
	Foliage (%)	Diet cubes (%)	Diet-filled cups (%)
NJSS-APHIS	96.6	100	85.7
NJSS-A-F	100	96.6	—
QC	96.6	90.0	69.6
MASS	100	86.6	0
VA	100	73.3	0

$N = 30$ for the foliage and diet cube treatments. N ranged from 21 to 24 in the diet-filled treatment and NJSS-A-F was not included in this treatment. Populations in this experiment included wild populations (QC, Quebec City, Quebec, Canada; MASS, Milford, Massachusetts; and NJ, Blairstown, New Jersey) and laboratory strains (NJSS-APHIS, New Jersey Standard Strain reared at an APHIS Laboratory; NJSS-FS, New Jersey Standard Strain reared at a Forest Service Research Center; and NJSS-A-Foliage, the APHIS strain reared on foliage for three generations).

Accordingly, survival was significantly different based on diet (logistic regression Wald $\chi^2 = 7.36$; df = 1, 5; $P = 0.007$) and marginally different between populations (Wald $\chi^2 = 9.43$; df = 4, 5; $P = 0.051$). Survival was lowest in the diet-filled treatment for the NJSS-APHIS and QC populations, with no larvae from MASS or VA surviving to pupation when reared in the filled cups.

Growth during the first stage of the experiment was more rapid on foliage, with larvae attaining masses from 1.9- to 3.7-fold higher than on artificial diet (Fig. 2). Accordingly, the effect of diet treatment was highly significant ($F = 941.7$; df = 1, 48; $P < 0.0001$). We also found significant differences between populations ($F = 25.5$; df = 4, 48; $P < 0.0001$), with laboratory strains at larger sizes than the wild populations. Additionally, the population effect interacted with diet treatment in our analysis of mass after stage 1 ($F = 5.81$; df = 4, 48; $P = 0.0007$). The effect of diet treatment on relative growth rate over the second stage of the experiment was highly dependent on population (population × diet interaction: $F = 10.94$; df = 7, 350; $P < 0.0001$; Fig. 3). During the second stage, the laboratory strains and the QC population had the highest growth on artificial diet in cubes, while the other two wild populations maintained higher growth on foliage. All wild populations had the lowest growth in diet-filled cups.

Similarly, the effect of diet treatment on pupal mass depended on population (interaction effect for females: $F = 5.45$; df = 4, 112; $P = 0.0005$; Fig. 4A; interaction effect for males: $F = 16.07$; df = 4, 129; $P < 0.0001$; Fig. 4B). For male pupae, the two laboratory strains and the QC population ultimately had similar pupal masses whether reared on foliage or diet cubes, but the MASS and the VA populations showed reduced pupal mass on diet cubes. Female pupae showed the same pattern, with the one notable difference that the mass of the NJSS-APHIS strain was actually lower on foliage compared with the diet cube treatment. Across treatments and populations, sex ratio averaged 47.0 ± 9.1% SD females.

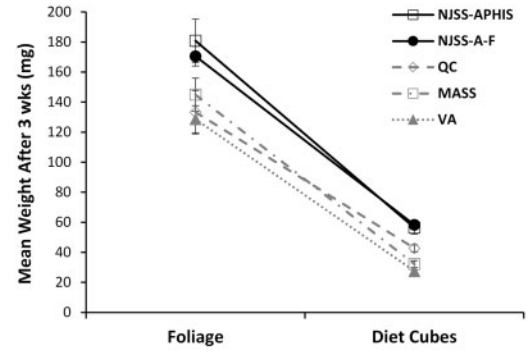

Fig. 2. Larval mass after the first 3 wk of development in a growth chamber under a cycling temperature regime with both foliage and artificial diet treatments. Only two diet treatments were tested during the first stage of the experiment, when larvae were group reared in containers. Populations in this experiment included wild populations (QC, Quebec City, Quebec, Canada; MASS, Milford, Massachusetts, and VA, Manassas, Virginia) and laboratory strains (NJSS-APHIS, New Jersey Standard Strain, reared at an APHIS Laboratory and NJSS-A-Foliage = the APHIS strain reared on foliage for three generations).

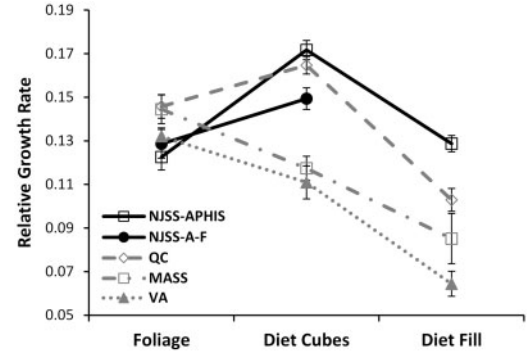

Fig. 3. Relative growth rate during the second stage of the growth chamber experiment comparing foliage and diet calculated from $[\ln(m_2) - \ln(m_1)]/t$. The diet cube treatment was split to include a treatment where larvae spent the remainder of the experiment in cups filled with artificial diet. Populations in this experiment included wild populations (QC, Quebec City, Quebec, Canada; MASS, Milford, Massachusetts; and VA, Manassas, Virginia) and laboratory strains (NJSS-APHIS, New Jersey Standard Strain reared at an APHIS Laboratory, and NJSS-A-Foliage, the APHIS strain reared on foliage for three generations).

In the common environment used for the third experiment, developmental rate was faster on foliage compared with artificial diet cubes, with larvae completing development on average 10.1 d faster on foliage. Accordingly, the effect of diet was significant for both sexes (females: $F = 107.7$; df = 1, 112; $P < 0.0001$; Fig. 4C; males: $F = 248.7$; df = 1,129; $P < 0.0001$; Fig. 4D). The main effect of population was also significant for both sexes (females: $F = 15.9$; df = 1, 112; $P < 0.0001$; males: $F = 24.6$; df = 1, 129; $P < 0.0001$), with laboratory strains and the QC population developing 6.3 d

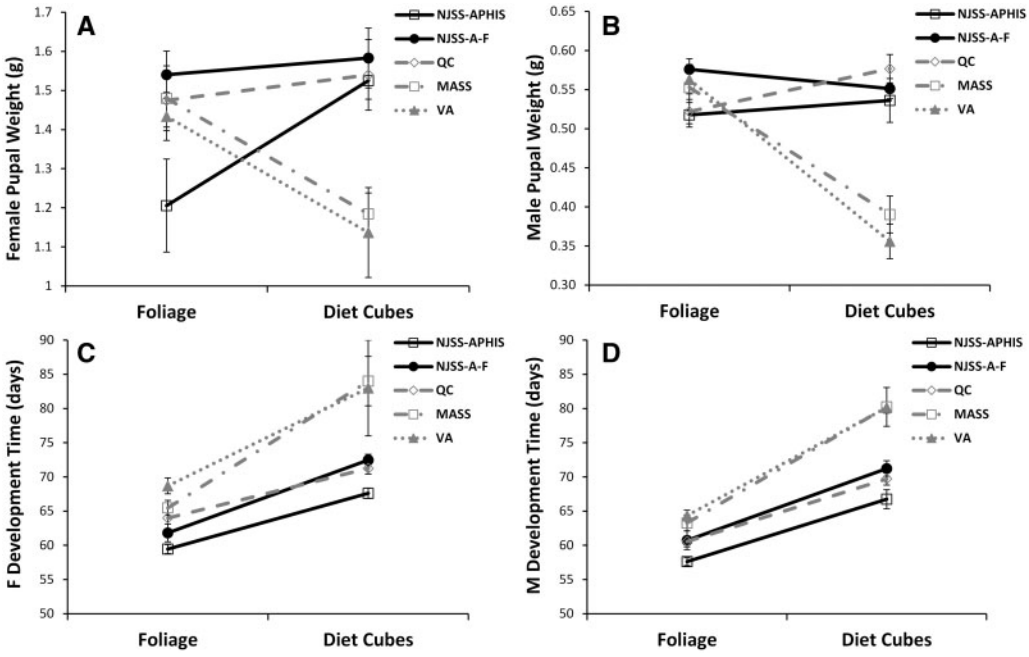

Fig. 4. Differences in pupal mass for female (A) and male (B) gypsy moths and differences in developmental time for females (C) and males (D) in a growth chamber experiment using a fluctuating temperature regime comparing foliage and diet. The diet-filled treatment was excluded because survival was too low for some populations. Populations in this experiment included wild populations QC, Quebec City, Quebec, Canada; MASS, Milford, Massachusetts; and NJ, Blairstown, New Jersey) and laboratory strains (NJSS-APHIS, New Jersey Standard Strain reared at an APHIS Laboratory; NJSS-FS, New Jersey Standard Strain reared at a Forest Service Research Center; and NJSS-A-Foliage, the APHIS strain reared on foliage for three generations). Female and male are abbreviated as F and M, respectively, in the y-axis label for graphs (C) and (D).

faster on average than the MASS and VA wild populations. The interaction between population and diet on developmental time was significant for males ($F = 2.63$; df = 4, 129; $P = 0.038$) but not for females ($F = 2.41$; df = 4, 112; $P = 0.053$).

Discussion

Experiments that rear insects on foliage can be extremely time consuming, costly, and often require precise timing with host plant phenology. Methods for insect rearing on artificial diet and the availability of laboratory strains have enabled a wide range of studies that could otherwise be infeasible. For gypsy moth, this work includes identification and testing of natural enemies, quantifying life history parameters, reproductive biology, developmental rates, and identification of the gypsy moth sex pheromone. The management outcomes of this work include development of the gypsy moth life stage model to predict life cycle phenology and formulation of a synthetic disparlure for use in monitoring traps and creation of mating disruption products (e.g., Bierl et al. 1970, Doane and McManus 1981, Gould et al. 1990, Gray 2004, Thorpe et al. 2008). For many ecologically important traits, the performance of laboratory strains can differ from wild populations and the use of artificial diet may not reflect growth and development on natural diets in some contexts. For gypsy moth, we found consistent differences in performance across populations and diets, an important consideration for future studies examining geographic variation or that use laboratory strains or artificial diets to extrapolate to wild populations.

The enhanced performance of gypsy moth laboratory strains is well-known. Several studies in gypsy moth and other insect systems have illustrated how artificial selection in laboratory colonies for increased production can result in insects with altered life history parameters (King and Leppla 1984). A review by Keena and Odell (1994) found that the NJSS develops faster, grows larger, and has increased reproduction compared with wild strains when mass reared on artificial diet. Our experiments also found large population differences in survival, growth, and development when rearing on artificial diet, with laboratory strains clearly outperforming wild populations. This is consistent with the results from diet switching experiments between artificial diet and northern red oak performed using NJSS-APHIS and a wild population from New York (Hajek 1989). Wild populations may have difficultly metabolizing components of the artificial diet or have reduced conversion efficiencies compared with laboratory strains selected for optimal growth on artificial medium (Lindroth et al. 1991). Future studies could benefit from detailed studies of consumption and assimilation efficiency to differentiate between the suite of potential physiological and behavioral responses to diet variation.

An encouraging result from our experiments is that the developmental differences between laboratory strains and wild populations were much smaller when larvae were reared on optimal host foliage. Many studies have compared the response of a single population to a wide variety of foliage diets, both using laboratory strains or a wild collected population (see studies cited by Liebhold et al. 1995), but fewer studies have compared the response across populations. Our results have specific implications for phenological studies and indicate that seasonal developmental trajectories based on laboratory strains can mirror wild populations under natural conditions.

Perhaps the most surprising outcome of our study was the variation among wild populations in performance on artificial diet. In our studies, field populations collected from New Jersey, Massachusetts, and Virginia developed poorly on artificial diet, while the population from Quebec performed nearly as well or even equivalent to different strains of NJSS when reared on diet cubes. Leonard (1968b) noted that gypsy moth larvae hatching from egg masses collected from Quebec developed more rapidly than larvae from Connecticut and speculated that these differences may represent an adaption for faster development in colder climates (i.e., "metabolic cold adaptation;" Addo-Bediako et al. 2002). The link between adaptive traits in more northern populations and higher performance on artificial diet is unclear, although the gypsy moth is known to have genetic variation in diet breadth (Rossiter 1987). Alternatively, this population could be an anomaly or experienced inadvertent selection during rearing. This population has been used in other comparative studies and has been consistently high performing (D. P., unpublished data). Future studies using additional population replicates from the region would clarify among these hypotheses.

The rapid development of larvae on foliage likely reflects our choice of northern red oak, a preferred host tree, which is often cited as one of the most optimal host trees for gypsy moth growth and development (Liebhold et al. 1995). Pupal masses obtained from cut foliage in our study are very similar to those obtained in the field from larvae reared directly on living red oak trees (e.g., Raupp et al. 1988, Stoyenoff et al. 1994). In the comparison experiment, growth was rapid across all populations on early season foliage, which is high in nitrogen and water content and low in leaf toughness (Hough and Pimentel 1978). Seasonal changes in foliage toughness and quality can reduce growth rates of caterpillars feeding on later season foliage (Feeny 1970, Hajek 1989). A decline in growth rate because of older larvae feeding on mature foliage was seen during the second stage of the comparison experiment. The laboratory strains and Quebec population experienced higher growth rates on diet cubes during this period, illustrating that artificial diet can be nutritionally superior to mid- and late season foliage for populations that perform well on diet (Hajek 1989).

Despite potentially limited genetic diversity in North American gypsy moth populations, especially the laboratory strain, shifts in population performance due to diet can occur over short time periods (Rossiter 1991), as evidenced by the results from the NJSS-A-Foliage group. Originally sourced from the NJSS-APHIS strain, the NJSS-A-Foliage group experienced three generations of rearing in a standardized array on a northern red oak foliage. In comparison to the larvae straight from laboratory conditions, the NJSS-A-Foliage group had intermediate growth rates in both experiments using artificial diet. This result suggests that selecting laboratory strain larvae that also developed well on foliage resulted in small changes in performance on artificial diet. We expect that the reverse scenario can also occur, where rearing wild populations on diet for multiple generations would quickly select for those capable of thriving on artificial diet and could potentially shift trait performance.

When rearing wild populations on artificial diet, our two experimental venues differed from mass-rearing protocols in important ways. Our first experiment used a constant 28°C rearing temperature. While this temperature has been shown to result in the fastest development times for gypsy moth larvae (Logan et al. 1991), it may not result in the highest pupal mass or fecundity and protocols for mass rearing use temperatures between 25 and 26°C (Singh and Moore 1985). The increased temperature in our experiment and its effects on artificial diet quality may have contributed to lower pupal masses than obtained in other studies (Doane and McManus 1981). However, our results are still valuable for their between population comparisons under the same rearing conditions. We also tested the difference between filling containers with artificial diet compared with providing cubes of artificial diet at regular intervals and we found much lower survival and growth from the filled container treatment. This result was consistent across all populations, with none of the MASS or VA larvae reaching pupation in filled cups. This treatment was originally intended to mimic the mass-rearing protocol for gypsy moth, where eggs or newly hatched larvae are placed in a 180-ml cup containing 100 g of artificial diet and left to develop until pupation in 4–5 wk without changing the medium (General Procedure for Rearing Gypsy Moth on Artificial Diet, APHIS Otis Lab, H. Nadal, unpublished). In practice, our filled cup treatment differed in several ways from this protocol that may have enhanced desiccation and reduced diet quality. Our experiment included a cycling temperature regime to maintain caterpillar development in line with rates of foliage maturation, which may have affected diet differently than a stable rearing temperature. We checked on larvae daily and removed individuals for weighing, likely increasing the exposure and drying of the diet. We also used a smaller 75-ml cup, and thus the diet had a higher surface to volume ratio. Finally, we reared larvae individually in the second stage of the experiment with filled cups. Previous work has demonstrated that gypsy moth larvae have increased survival in groups (Leonard 1968a), and more individuals feeding would expose more diet under the surface layer. Barbosa and Capinera (1977) also noted high larval mortality (~80%) in a wild population reared on artificial diet used for

longer periods and found the provision of fresh diet substantially improved survival rates. Providing artificial diet in cubes has been used in many caterpillar rearing experiments and has the additional benefit of reducing contamination (Singh and Moore 1985). While an inefficient technique for mass rearing, our results indicate that providing artificial diet in cubes is a superior method for small-scale laboratory research, particularly when using wild populations.

Understanding population differentiation in developmental traits, particularly at invasion fronts, is important for making predictions about future spread and is directly relevant to site-specific gypsy moth management decisions. The leading edge of the gypsy moth invasion spans climatic extremes, recently reaching Cook and Lake counties in Minnesota to the north (United States Code of Federal Regulations, Title 7, Chapter III, Section 301.45–3), and persisting in Virginia and North Carolina, where unexpected patterns of range stasis and contraction are occurring (Tobin et al. 2014). Comparisons across wild populations are critical for understanding these responses and testing the potential for life history adaptations at the leading edge of the invasion. Laboratory rearing methods and artificial diet can enable a wide range of research questions using wild populations without the complication of synchronizing development with host foliage. However, our work indicates that the response of wild populations to artificial diet can be variable, complicating the interpretation of population comparisons in controlled experiments. Quantifying the response of wild populations on both natural and artificial diets is an important first step for studies in experimental venues.

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