Evolutionary Divergence in Thermal Sensitivity and Diapause of Field and Laboratory Populations of *Manduca sexta*

Joel G. Kingsolver*
Annemarie Nagle
Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599

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ABSTRACT

The tobacco hornworm *Manduca sexta* has been an important model system in insect biology for more than half a century. Here we report the evolutionary divergence in thermal sensitivity and diapause initiation between field and laboratory populations that were separated for more than 35 yr (>240 laboratory generations) and that are descendants from the same field populations in central North Carolina. At intermediate rearing temperatures (20°–25°C), mean body size was significantly larger and development time significantly faster in the laboratory than in the field populations. At higher temperatures (30°–35°C), these mean differences between populations were reduced or eliminated, and larval survival at 35°C was significantly lower in the laboratory population than in the field population. F_1 crosses had survival and development time to wandering similar to the field population times at both 25° and 35°C; body mass at wandering for F_1 crosses was intermediate compared with that of the field and laboratory populations. Comparisons with earlier field and laboratory studies suggest evolutionary reductions in thermal tolerance and performance at high temperatures in the laboratory population. The critical photoperiod initiating diapause in field populations in North Carolina did not change detectably between the 1960s and 2005. In contrast, the laboratory population has evolved a reduced tendency to diapause under short-day conditions, relative to the field population.

Introduction

The tobacco hornworm *Manduca sexta* has been an important model system in integrative biology for more than half a century. For example, *Manduca* research has been fundamental to our understanding of the physiology and hormonal control of molting and metamorphosis (Nijhout 1994; Gilbert et al. 2000; Riddiford et al. 2003; Truman et al. 2006). Similarly, growth and feeding, foraging behavior, nutritional physiology, thermal biology, secondary plant chemistry, and natural enemies have been explored in detail for *Manduca* (Casey 1976; Reynolds and Nottingham 1985; Reynolds et al. 1985, 1986; Stamp and Horwath 1992; Stamp and Skrobola 1993; Stamp and Yang 1996; Kingsolver and Woods 1997, 1998; Woods and Kingsolver 1999; Petersen et al. 2000). These studies of *Manduca* have been facilitated by its pest status, rapid growth, large size, and ease of laboratory rearing.

The first large-scale rearing facility for *M. sexta* (the Yamamoto strain) was established in Raleigh, North Carolina, at North Carolina State University (NCSU) in the 1960s (Yamamoto and Fraenkel 1960; Yamamoto et al. 1969; Yamamoto 1974), initiated from field collections in tobacco plots at the NCSU Research Station in Clayton, North Carolina. Two USDA facilities in Fargo, North Dakota, and Beltsville, Maryland, were later established using material from the NCSU colony. To our knowledge, all current major scientific laboratory colonies of *M. sexta* are ultimately derived, directly or indirectly, from the NCSU colony. Under standard *Manduca* rearing conditions, this represents more than 240 generations in laboratory conditions since the colonies were first established.

Many studies have documented evolutionary domestication or adaptation to laboratory conditions in insects (Service and Rose 1985; Harshman and Hoffmann 2000; Matos et al. 2000). Recent studies with *Manduca* show that mean maximal larval mass has increased by about 50% in a laboratory population between 1971–1973 and 1999–2000 (D'Amico et al. 2001). This evolutionary change in size was largely the result of changes in growth rate, critical weight, and the timing of hormonal secretion during the final (fifth) larval instar (D'Amico et al. 2001; Davidowitz et al. 2003, 2004, 2005; Davidowitz and Nijhout 2004). However, evolution of other aspects of *Manduca* biology during domestication has not been explored (Kingsolver 2007).

Within its geographical range in the southeast and southwest of North America, *Manduca sexta* larvae typically experience a wide range of environmental and body temperatures during growth and development, including maximal diurnal temperatures exceeding 35°C during summer and subfreezing temperatures during winter (Casey 1976). *Manduca* overwinters in a pupal diapause that is initiated by day length (Rabb 1966). By contrast, standard rearing conditions for *Manduca* include

* Corresponding author; e-mail: jgking@bio.unc.edu.

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constant temperatures (25°–27°C) and long day lengths (14–16 h light).

In this article, we examine the thermal sensitivity of survival, growth, and development and the critical photoperiod for diapause initiation in a laboratory colony and in the field population of *M. sexta* (Clayton, NC) from which the laboratory colony was originally derived. We also evaluate the thermal sensitivity of survival, growth, and development for F_1 crosses between field and laboratory animals. We compare these results with those in previous studies of field populations in the 1960s and 1970s and of laboratory colonies in the 1970s and 1980s. Our results demonstrate how thermal sensitivity and diapause initiation have diverged between laboratory colonies and field populations of *Manduca* within the past 40 yr.

Material and Methods

Our studies used *Manduca sexta* from two sources. The laboratory population was taken from a laboratory colony maintained under standard larval rearing conditions (artificial diet, constant 25°C, 15L : 9D photocycle) by L. Gilbert and colleagues at the University of North Carolina (UNC) for more than 25 yr. As described above, this colony was ultimately derived from the NCSU colony established during the 1960s. The field population was established from eggs collected in tobacco fields at the NCSU Research Station in Clayton, North Carolina (Sampson County); this region was the original field source of the NCSU laboratory colony (see above). All experiments were initiated with newly hatched first-instar larvae during the summer and fall of 2005, and larvae were reared in individual petri dishes. Larvae were reared in environmental chambers (Percival 36-VL) on standard *Manduca* diet (Riddiford 1967) with the addition of small amount of dried tobacco leaf (8.3% dry weight of diet). We found that this addition facilitated consistent feeding and growth by the field larvae. Field-collected eggs were reared through one generation in the laboratory under standard conditions (25°C, 16L : 8D, artificial diet) before use in the experiments.

For the thermal studies, larvae were reared at one of five constant temperature treatments—15°, 20°, 25°, 30°, or 35°C—in long-day conditions (16L : 8D) until wandering during the last larval instar. Wandering was defined by the cessation of feeding and by movement of the larva around the rim of the Petri dish (Nijhout and Williams 1974). Sample sizes (no. individuals at hatching) for the field population were 43 (15°C), 30 (20°C), 28 (25°C), 29 (30°C), and 36 (35°C); sample sizes for the laboratory population were 28 (15°C), 24 (20°C), 30 (25°C), 25 (30°C), and 30 (35°C). After wandering, larvae were placed in cedar blocks in the laboratory (~25°C) for pupation. Survival, development time, mass at wandering, pupation, and eclosion were recorded. Here we focus on survival, development time, and mass at wandering; analyses of measurements at pupation yield qualitatively similar results. Growth and developmental trajectories for the 20°, 25°, and 30°C treatments of the field and laboratory populations were described by Kingsolver (2007).

Survival was considered a binomial variable and modeled using analysis of deviance, with temperature treatment and population (laboratory or field) as fixed effects; development time and mass at wandering were modeled using ANOVA, with temperature treatment and population as fixed effects. All statistical analyses were performed using S-Plus 6.2 statistical software.

We also measured survival, growth, and development at two constant temperatures (25°C and 35°C) for F_1 crosses between field and laboratory individuals. For these studies, 8–10 virgin field females (F♀) and 8–10 virgin laboratory males (L♂) were placed together in 1-m^3 mating cages with several tobacco plants; eggs from this cross (F♀ × L♂) were collected and reared after hatching as described above. The reverse cross (L♀ × F♂) was also performed. Samples sizes for the F♀ × L♂ cross were 40 (25°C) and 40 (35°C); sizes for the L♀ × F♂ cross were 40 (25°C) and 37 (35°C). Survival, development time, and mass at wandering were measured and analyzed as above.

For the critical photoperiod studies, larvae were reared until wandering at a constant (25°C) temperature at one of four day length treatments: 15L : 9D, 14L : 10D, 13L : 11D, or 12L : 12D. Twenty-five to 30 larvae were used for each population at each day length. After wandering, larvae were placed with soil in individual plastic cups for pupation in the laboratory (25°C). Under these conditions, nondiapausing pupae will eclose within 20–35 d, whereas diapausing pupae will take 60 d or more to eclose (Rabb 1966, 1969). We used duration of the pupal stage to distinguish diapausing (>60 d) from nondiapausing (<35 d) individuals; no individuals in the study had pupal durations

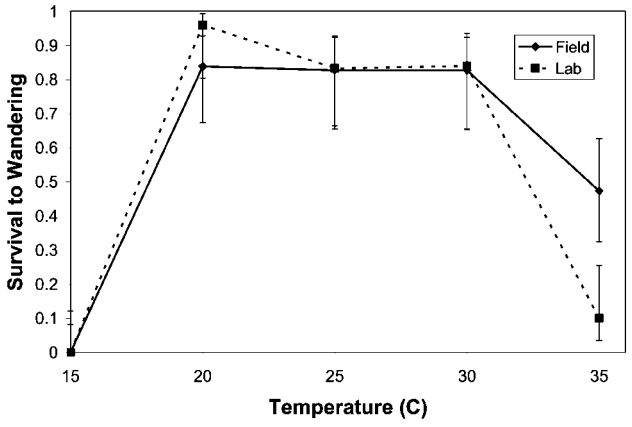

Figure 1. Mean larval survival from hatching to wandering as a function of rearing temperature (in °C) for field (*solid line*, *squares*) and laboratory (*dashed line*, *diamonds*) populations of *Manduca sexta*; 95% confidence intervals are indicated.

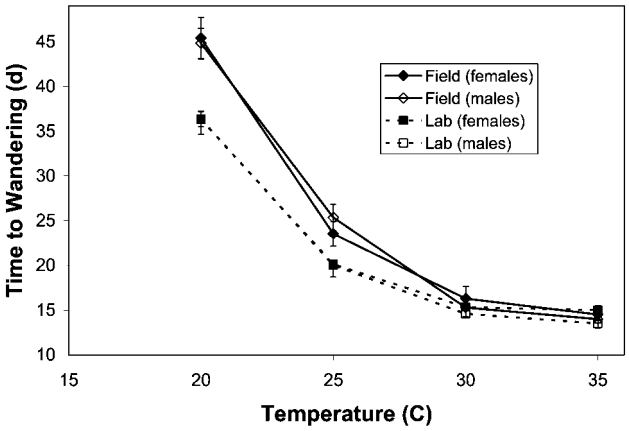

Figure 2. Time to wandering as a function of rearing temperature (in °C) for field (*solid lines*) and laboratory (*dashed lines*) populations of *Manduca sexta*. Mean ± 1 SE are indicated. Data for females (*filled symbols*) and males (*open symbols*) are given separately.

between 35 and 60 d. Diapause was considered as a binomial variable and modeled using analysis of deviance, with day length treatment and population (laboratory or field) as fixed effects.

Results

Both laboratory and field larvae had high survival to wandering (>80%) at temperatures between 20° and 30°C and had no survival at 15°C (Fig. 1). At 35°C, laboratory larvae had low survival (10%), whereas field larvae had intermediate survival (47%). Analysis of deviance detected significant effects of temperature treatment ($\chi^2 = 66.5$, $P \ll 0.001$) and of the interaction between temperature and population ($\chi^2 = 12.2$, $P = 0.007$) on the probability of survival to wandering, but there was no significant effect of population ($\chi^2 = 2.1$, $P = 0.14$); the significant interaction between temperature and population resulted largely from the difference in mean survival between populations at 35°C.

Mean development time to wandering declined with increasing temperatures between 20° and 30°C for both laboratory and field populations (Fig. 2). At lower (20°–25°C) temperatures, time to wandering was substantially longer in field larvae than in laboratory larvae but converged to similar times at higher temperatures. Wandering times were similar for males and females. ANOVA detected significant effects of temperature ($F_{3,149} = 428.4$, $P \ll 0.001$), population ($F_{1,149} = 49.3$, $P \ll 0.001$), and temperature × population interactions ($F_{3,149} = 9.1$, $P < 0.001$) on time to wandering but no effect of sex ($F_{1,149} = 0.03$, $P = 0.85$).

Mean mass at wandering (maximum larval mass) declined with increasing temperature in both populations (Fig. 3), with the greatest declines for laboratory larvae between 30° and 35°C. At temperatures below 35°C, laboratory larvae were substantially larger than field larvae. For the laboratory population, females were larger than males at temperatures between 20° and 30°C, whereas in the field population, females and males were similar in size except at 20°C. ANOVA revealed significant effects of temperature ($F_{3,149} = 46.7$, $P \ll 0.001$), population ($F_{1,149} = 98.2$, $P \ll 0.001$), sex ($F_{1,149} = 6.3$, $P = 0.013$), and temperature × population interaction ($F_{3,149} = 2.8$, $P = 0.041$) on mass at wandering.

For the two F_1 crosses, survival to wandering was not significantly affected by either rearing temperature or the direction of the cross (Fig. 4). Mean survival at 25°C for the F_1 crosses was similar to or slightly less than that for the field and laboratory populations, whereas at 35°C, it was greater than that for either field or laboratory population. Development time to wandering was significantly affected by temperature ($F_{1,103} = 242.0$, $P \ll 0.001$) but not by sex ($F_{1,103} = 0.630$, $P = 0.429$) or the direction of the cross ($F_{1,103} = 2.7$, $P = 0.105$). Mean development time at 25°C for the F_1 crosses was similar to slightly greater than that for the field population (Fig. 5); at 35°C, mean development times for all groups converged. There were significant effects of temperature ($F_{1,103} = 142.3$, $P \ll 0.001$), sex ($F_{1,103} = 8.2$, $P = 0.005$), and the direction of the cross ($F_{1,103} = 4.3$, $P = 0.040$) on mass at wandering (Fig. 6). Mean mass at wandering was greater for the F♀ × L♂ cross than for the L♀ × F♂ cross for both females and males. Mean masses for the F_1 crosses were intermediate to those between the field and laboratory populations (Fig. 6).

The field population showed a rapid transition from a high proportion (>95%) of diapausing pupae at 12-h and 13-h day lengths to a low proportion (<5%) at longer day lengths (14 h or 15 h light; Fig. 7), resulting in a critical photoperiod of 13.5 h. The relationship between diapause and day length in the field population was very similar to that reported by Rabb

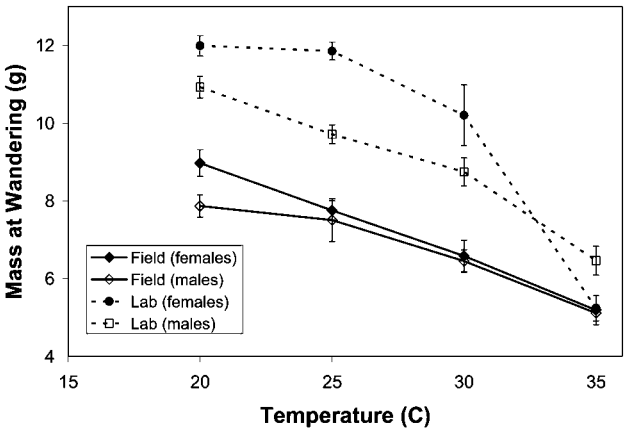

Figure 3. Body mass at wandering as a function of rearing temperature (in °C) for field (*solid lines*) and laboratory (*dashed lines*) populations of *Manduca sexta*. Mean ± 1 SE are indicated. Data for females (*filled symbols*) and males (*open symbols*) are given separately.

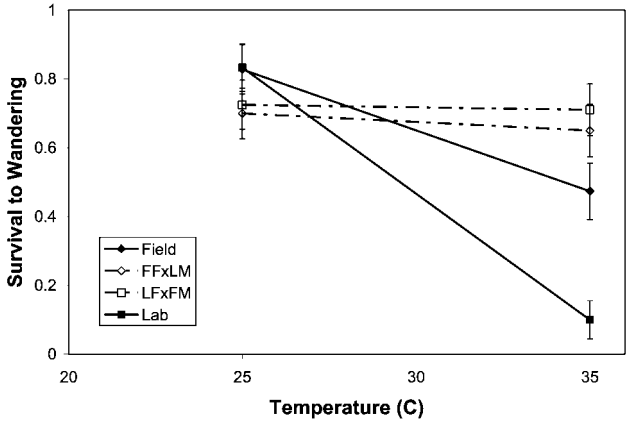

Figure 4. Mean (±1 SE) larval survival from hatching to wandering as a function of rearing temperature (in °C) for F_1 crosses (*dashed lines*) between field and laboratory *Manduca sexta*. Field females × lab males (FF × LM): open squares; lab females × field males (LF × FM): open diamonds. Data for laboratory (*filled squares*) and field (*filled diamonds*) populations are included for comparison (see Fig. 1).

(1966, 1969) for North Carolina field populations of *Manduca sexta* in the late 1960s (Fig. 7). In contrast, the laboratory population showed a lower proportion of diapausing pupae at 13-h day length (56.6%) compared with the field population (96.3%). Analysis of deviance detected significant effects of day length ($\chi^2 = 165.7$, $P \ll 0.0001$) and population ($\chi^2 = 12.2$, $P = 0.0005$), with a marginally significant interaction between day length and population ($\chi^2 = 5.8$, $P = 0.055$).

Discussion

Our results show that the laboratory and field populations of *Manduca sexta* have diverged substantially in size and development time under standard laboratory rearing conditions (25°C). Under these conditions, laboratory individuals were 25%–50% larger in maximum larval mass and had 15%–20% faster development times than field individuals (Figs. 1, 2). In addition, there was a striking sexual dimorphism in size in the laboratory population, with females more than 20% larger in maximum larval mass than males; by contrast, males and females were similar in size in the field population. D'Amico et al. (2001) showed that for a *Manduca* laboratory colony in 1971–73 (soon after the initial establishment of the colony in the late 1960s), mean maximum larval mass was 7.8 g—very similar to that observed for our current field population (mean = 7.9 g). This suggests that the divergence in size between laboratory and field populations during the past 40 yr was largely due to increases in mean size in the laboratory populations rather than changes in size in field populations. These changes are probably due to inadvertent selection in the laboratory for large size and rapid growth rates while feeding on artificial diet (Rabb 1971; D'Amico et al. 2001; Davidowitz et al. 2003, 2004, 2005; Davidowitz and Nijhout 2004).

A main result of our studies is the evolutionary divergence in thermal sensitivity of development time, size, and survival between laboratory and field populations (Figs. 1–3). Neither the laboratory nor the field population was able to survive or maintain consistent growth at 15°C. The mean development time was longer for the field population than for the laboratory population at 20°–25°C, but this difference disappeared at higher temperatures (30°–35°C). The relatively longer development times of the field population at lower temperatures is associated with variation in the number of larval instars; the frequency of field individuals that had six larval instars decreased from 69% at 20°C, 48% at 25°C, and 17% at 30°C (Kingsolver 2007). In contrast, laboratory individuals had the typical five larval instars at all rearing temperatures. The consequence of the additional instar was to increase the development time (time to wandering), particularly at lower temperatures. The additional instar in field individuals probably occurs because the artificial diet used in these studies represents a novel, low-quality food resource for the field population, whereas the laboratory population is well-adapted to the artificial diet (Kingsolver 2007). Of course, thermal sensitivity of growth and development in insect larvae may depend on food resource (Kingsolver et al. 2006).

The big difference in mass at wandering between laboratory and field populations at lower temperatures (20°–25°C) largely disappeared at 35°C. In addition, larval survival at 35°C was reduced in the laboratory population relative to the field population. These results suggest a reduction in adaptation to high-temperature conditions in the laboratory population relative to

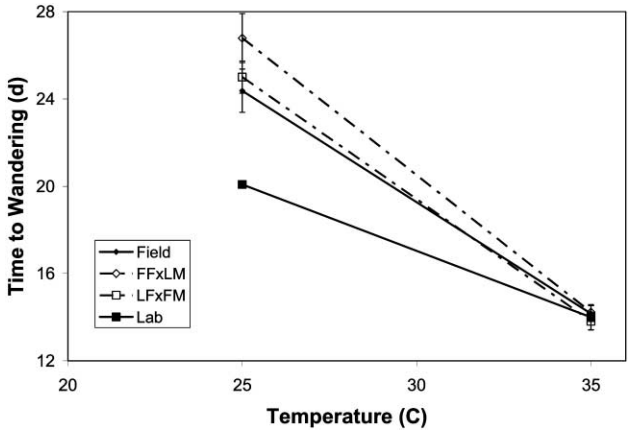

Figure 5. Mean (±1 SE) time to wandering (in d) as a function of rearing temperature (in °C) for F_1 crosses (*dashed lines*) between field and laboratory *Manduca sexta*. Field females × lab males (FF × LM): open squares; lab females × field males (LF × FM): open diamonds. Data for laboratory (*filled squares*) and field (*filled diamonds*) populations are included for comparison (see Fig. 2).

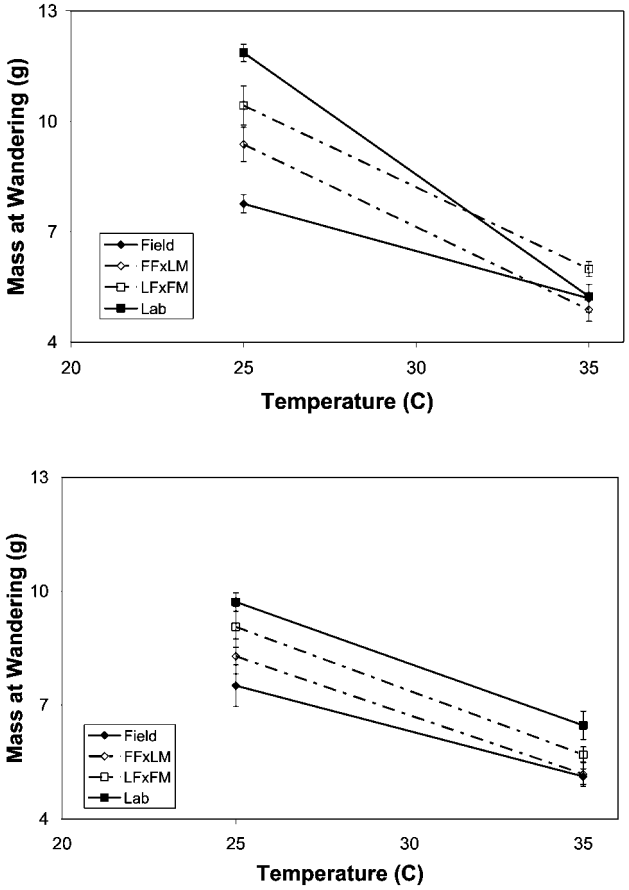

Figure 6. Mean (±1 SE) mass to wandering (in g) as a function of rearing temperature (in °C) for F_1 crosses (*dashed lines*) between field and laboratory *Manduca sexta*. Field females × lab males (FF × LM): open squares; lab females × field males (LF × FM): open diamonds. Data for laboratory (*filled squares*) and field (*filled diamonds*) populations are included for comparison (see Fig. 3). *Top*, females; *bottom*, males.

our field population from North Carolina (Krebs et al. 2001). In July–August in central North Carolina, maximum daily air temperatures frequently exceed 35°C. A field study of *M. sexta* in the Mojave Desert (Palmdale, CA) showed that caterpillar body temperatures exceeding 35°C were typical in summer, midday conditions (Casey 1976). Laboratory studies of the Mojave population in the early 1970s indicated relatively high growth rates and survival for *M. sexta* reared on tobacco leaves at 35°C (Casey 1977). Conversely, studies of a laboratory colony in the early 1980s reported high larval mortality for *M. sexta* reared from hatching at 35°C (Reynolds and Nottingham 1985). Collectively, these results suggest evolutionary reductions in tolerance and performance at higher temperatures in laboratory colonies, probably reflecting their lack of exposure to higher temperatures during domestication.

F_1 crosses between the field and laboratory populations yielded several interesting patterns. Mean body mass at wandering for F_1 individuals was intermediate between those for field and laboratory populations of both males and females; F_1 offspring with laboratory mothers were larger than those with field mothers, indicating a maternal or sex-linked contribution to body size. At standard lab temperatures (25°C), F_1 individuals had similar mean survival and slightly longer mean development times than the field population; there is little suggestion of either strong hybrid vigor or hybrid inferiority. We did not notice reduced hatching success in the crosses, although this was not quantified in our studies. In contrast, at high temperature (35°C), F_1 individuals had higher mean survival than either field or lab individuals. Further genetic analysis of high temperature tolerance may prove valuable.

Finally, our analyses detected no evolutionary changes in the critical photoperiod for diapause initiation in field populations of *M. sexta* from North Carolina between the 1960s (Rabb 1966, 1969) and 2005 (Fig. 3). Recent studies of pitcher plant mosquitoes in the eastern United States documented evolutionary changes in critical photoperiod during the past 3 decades, presumably as a response to recent climate warming (Bradshaw and Holzapfel 2001); however, greater evolutionary changes were detected in northern than in southern populations. Our laboratory population of *M. sexta* showed a significantly reduced tendency to diapause at shorter day lengths (13 h) compared with the field population. Apparently, continued exposure to long-day, nondiapause conditions has led to an evolutionary reduction in diapause tendency at shorter day lengths in the laboratory population. The mechanisms underlying this evolutionary change remain to be explored.

The evolutionary divergence of laboratory and field populations demonstrated here has two important implications for

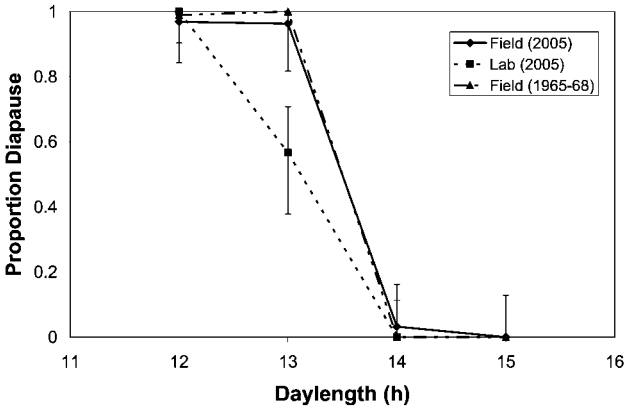

Figure 7. Mean proportion of diapausing pupae as a function of day length (no. h of light/day) for field (*solid line*, *squares*) and laboratory (*dashed line*, *diamonds*) populations of *Manduca sexta*. 95% confidence intervals are indicated. Data for a North Carolina field population of *Manduca sexta* from the 1960s (*broken line*, *triangles*) are also included (data from Rabb 1966, 1969).

the studies of *Manduca*. First, many studies have explored aspects of feeding, growth, diapause, and thermal sensitivity using laboratory colonies of *Manduca*. It is important to clarify whether these reflect adaptive responses to natural or to laboratory conditions. Second, these differences between laboratory and field populations of *Manduca* represent a useful resource for examining the genetic and mechanistic bases of variation in growth, development, and thermal sensitivity in this important model system. Genetic crosses and backcrosses between field and laboratory lines may be particularly valuable for this purpose.

Acknowledgments

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