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Genetic analysis of larval survival and larval growth of two populations of *Leptinotarsa decemlineata* on tomato

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Abstract

The genetics of adaptation to tomato in *Leptinotarsa decemlineata* (Say) were investigated in reciprocal F_1, F_2, and backcross populations generated from crosses between beetles from a tomato adapted population and from a population that was poorly adapted to tomato. Larvae from the parent and test populations were reared on tomato for four days, after which survivorship and larval weights were recorded. Most results indicate that differences in larval growth and survival on tomato between the parent populations are largely determined by autosomal, polygenic mechanisms, the inheritance of which involves a significant dominance component. However, results from F_2 crosses are not consistent with this conclusion. A significant difference in larval weights, but not in survival, between reciprocal F_1 populations in an analysis of combined data from four separate experiments suggests that maternal cytoplasmic effects may contribute to differences in larval performance on tomato between the adapted and unadapted populations. The unusual results obtained from F_2 crosses in this study are not atypical of results from previous studies of the genetics of adaptation to host plants by the Colorado potato beetle. Host plant adaptation by Colorado potato beetles may therefore involve unusual genetic mechanisms that are not easily assessed by classical Mendelian analysis.

Introduction

Although the host range of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), includes approximately 20 species within the family Solanaceae, local populations of *L. decemlineata* vary in their ability to use particular plant species as hosts (Hsiao, 1978; de Wilde & Hsiao, 1981; Hare & Kennedy, 1986; Harrison, 1987; Kennedy & Farrar, 1987; Horton et al., 1988; Franca et al., 1994; Lu & Logan, 1994a, b). Genetic variation within and among populations in the ability of *L. decemlineata* to use a particular plant as a host, as well as differences in the availability of particular host plants (or host plant arrays) contribute to observed patterns of host use (Hsiao, 1978; Hare & Kennedy, 1986; Horton et al., 1988; Franca et al., 1994; Lu et al., 1997).

Tomato, *Lycopersicon esculentum* Miller, is generally a poor host for most *L. decemlineata* populations (Bongers, 1970; Latheef & Harcourt 1974; de Wilde & Hsiao, 1981; Szentesi & Jermy 1993; Weber et al., 1995). However, in some areas *L. decemlineata* has adapted to use tomato as a host and has become an important pest on this crop (Schalk & Stoner, 1979; Kennedy et al., 1983).

Recently, Lu et al. (1997) documented that *L. decemlineata* larvae from a population collected from potato in Massachussetts exhibited greater survival and growth on tomato than larvae from a population collected from potato in New York. They documented that these differences were genetically based. In addition, they demonstrated that selection of the New York population on tomato over three generations resulted in increased larval survival and growth, whereas the Massachusetts population showed no response in either trait after four generations of selection on tomato, but showed a significant increase in larval growth after 12 generations of selection. Reciprocal F_1 crosses

between beetles from a Massachusetts population selected on tomato for 14 generations and a New York population that had not been selected, and reciprocal crosses between selected and unselected populations from Massachusetts provided clear evidence that the differences between the parent populations in these crosses were genetically based. Analysis of these F_1 offspring indicated that the improved larval survival and growth following selection were generally inherited as dominant traits, and there was no evidence of either sex linkage or cytoplasmic inheritance. However, F_1 crosses can overestimate dominance (Mather & Jenks, 1977) and provide no information on the number of genes coding for larval performance. The present study was undertaken to characterize more clearly the genetic basis for adaptation to tomato in the populations studied by Lu et al. (1997). By carrying our F_2 crosses as well as backcrosses to the unadapted parental strain, and analyzing traits of the progeny with quantitative genetic techniques (e.g., Lande, 1981; Lynch & Walsh, 1997), we expected to be able to estimate the number of genes that affected larval performance on tomato. These techniques estimate the number of genes based on how much more variable the growth of the F_2 and backcross offspring are compared to the larvae from the parental strains and the F_1 crosses (Lande, 1981). Because the environmentally induced variation among larvae should be the same for all offspring grown at the same time on the same hosts, regardless of their parentage, any additional variation among larvae from a specific cross can be attributed to genetic differences among offspring from that cross. While larvae from the parental strains are all expected to be homozygous for growth-related genes and the F_1 offspring are all expected to be heterozygous for these genes, the F_2 and backcross offspring should be segregating for all genes that differ between the two parental strains (for exceptions to this see Lande, 1981).

In the simplist hypothetical case in which only a single additively acting gene is involved in adaptation to tomato, three genetically based larval growth classes are expected among the F_2 offspring (i.e., homozygous unadapted, heterozygous, and homozygous adapted), and two growth classes are expected among the backcross offspring (i.e., homozygous unadapted and heterozygous). When weights of these two or three genetically based growth classes of offspring are pooled and analyzed statistically, the variation around the mean is expected to be larger for the F_2 and backcross offspring than for the parental or F_1 offspring, for which, in each case, there is only one genetic growth class. If two or more genes affect growth, distinct growth classes are unlikely to be observed. However, if there are only a few genes for growth on tomato that differ between the strains, the variation in growth among larvae of the F_2 and backcrosses still is expected to be larger than among F_1 larvae because they will vary in the percentage of 'tomato growth genes' that they inherit. As the number of genes involved in growth on tomato increases (e.g., above ten), most offspring will, by random independent assortment, have about the same total number of tomato growth genes, so the variability in growth of larvae of each F_2 and backross is expected to become more similar to that of the parental and F_1 larvae. However, because most F_2 larvae would lack adaptive alleles at some of the loci, while all of the F_1 larvae would have one adaptive allele at each locus (assuming fixation in the parents), the mean weight of F_2 larvae would be expected to be less than that of F_1 larvae if the adaptive trait is under dominant control. In this paper, we present results on growth and survival of larvae from a series of F_1, F_2, and backrosses that are not predicted by classical quantitative genetic theory. We also provide a survey of previously reported findings on the genetics of adaptation by Colorado potato beetle to host plants, which indicates that our results are not atypical.

Materials and methods

The beetles used in these experiments were descendants of at least 50 adults collected from each of two separate populations. One colony, designated the UM strain, was originally collected in Massachusetts and was provided to us by D. N. Ferro in 1990. The other, designated the NY strain, was collected in Suffolk County, New York in 1990. Beetles from the NY colony used as parents in crosses were maintained continuously on potato plants ('Kennebec') in a greenhouse. Depending on the experiment, beetles from the UM colony used as parents were maintained as larvae on either potato or tomato for varying numbers of generations as indicated below. In all instances the adult beetles were fed potato foliage. Voucher specimens have been deposited in the Museum of Entomology at North Carolina State University.

All experiments to evaluate performance on tomato of progeny from various crosses lasted four days and were conducted in greenhouses in which

1200 W mixed metal halide lamps augmented natural daylength to a minimum of 14 h. During the course of the experiments, greenhouse temperatures at plant height ranged from 18 to 35 °C. All plants were grown in Metro-Mix 220 growing medium (Grace Sierra Horticultural, Milpitas, CA) and watered as needed. Tomato plants were grown in 15 cm clay pots, fertilized with 5–7 g Osmocote fertilizer (14-14-14 N:P:K; A. H. Hummert Seed, St. Louis, MO) per pot, and used when 5–6 weeks old.

When specifying the origin of hybrid populations, the female parent is always listed first, followed by the male parent. For example, progeny of the cross UM ∗ NY were derived from matings between UM females and NY males. Each experimental population (parent, F_1, F_2 or backcross) was generated by pairing 30–50 virgin female beetles with equal or fewer mates and allowing them to feed and oviposit on potato. We discarded eggs laid in the first week to allow time for most beetles to initiate oviposition. Before each experiment, at least 20 egg masses (if possible) each containing 25 or more eggs were collected from each experimental population. Potato foliage was trimmed from around each egg mass and the egg masses were stored on filter paper in petri dishes at 27 ± 0.5 °C. Once the eggs began to hatch, we moistened the filter paper and held the petri dishes at room temperature for ≈20 h before transferring the neonates to tomato (cultivar 'Better Boy') plants, which were then covered by cages made from polyester organza. The bases of the cages were tightened around the stems of the plants. After four days on tomato, all live larvae were weighed on a Mettler-AE240 electronic balance, either singly or as a group depending on the specific experiment, and the number of living larvae recorded.

For all statistical analyses, we checked plots of residual variance and when appropriate performed data transformations as indicated below. When results were not different between the original and transformed data, we report only the analyses of the original data.

Hybridization in May 1992

F_1 Cross. In 1992, we prepared reciprocal F_1 hybrid populations using virgin beetles from UM and NY to determine the basic inheritance of differences between the UM and NY populations in larval survival and larval weight. Parent beetles from both populations had been maintained continuously on potato. We measured larval survival and weight after four days on tomato as indicated previously for neonates from each parental population (UM and NY) and each reciprocal F_1 population (UM ∗ NY and NY ∗ UM). One hundred larvae from each population were evaluated in 10 groups of 10 larvae per tomato plant in 10 cages (10 larvae per cage). We measured larval survival (%) and mean larval weight (mg) for each cage (total weight/total number of survivors) after four days. We conducted six replicates of this experiment over time. Thus, the experiment was a randomized complete block design of four populations (UM, NY, UM ∗ NY, and NY ∗ UM), six replications, and 10 cages per replication. A 2-way analysis of variance (ANOVA) was used to examine the data on larval survival and mean larval weight. The effects of population and replication were tested against the interaction between the two, and the interaction was tested against experimentwise error. Tukey's studentized range test was used to compare differences in larval survival and mean larval weight between the parental and F_1 populations, using PROC GLM (SAS Institute, 1989). No data transformation was necessary.

F_2 cross. Each reciprocal F_1 population was reared on potato and adults within each population were allowed to randomly mate to produce populations of F_2 larvae. Protocols were the same as in the F_1 experiment, except that larvae were weighed individually. We calculated mean larval weight and the coefficient of variation (CV) in larval weight for each cage. CV was preferred over variance because it is standardized by mean weight and therefore allows for comparisons of the relative variance in F_2 hybrid populations and both parental populations. If one gene or a few genes controlled larval weight, we would expect greater variance in the segregating F_2 population than in the F_1 or parental populations. This was a randomized complete block design of four populations, seven replications, and 10 cages per replicate. ANOVAs for the F_2 data and comparisons of differences among populations in larval survival and mean larval weight were the same as in the F_1 experiment. In ANOVA for the CV data, we tested the main effects and the interaction against experimentwise error.

Backcross of F_1 to NY parents. Reciprocal F_1 backcrosses to the NY parental population were prepared and the progeny were evaluated as in the F_1 cross described above. In these evaluations, the UM and NY parental populations were included as controls. Protocols were again the same as in the F_1 experi-

ment, except that there were eight replications, and the UM and NY parent populations were not included as controls in the first three replications. Therefore, this was an unbalanced randomized block design of six populations (parents: UM, NY; reciprocal backcrosses: UMNY $*$ NY, NY $*$ UMNY, NYUM $*$ NY, and NY $*$ NYUM), 5–8 replications, and 10 cages per replicate. ANOVAs and comparisons of differences between the parental and backcross populations in larval survival and mean larval weight were conducted as in the F_1 experiment.

Hybridization in May 1993

Another set of reciprocal F_1, F_2, and backcross populations were generated as in 1992. To compare variances in F_2 populations with those the F_1 and parental populations, we tested all parental, F_1, F_2, and backcross populations simultaneously. Between May and June, after producing F_1 offspring needed to generate F_2 and backcross populations, the parents of the F_1 were stored in an incubator at 12.5 °C. When the initial group of F_1 offspring had matured, mated, and begun to produce F_2, and backcross offspring, the original parents were returned to normal rearing conditions on potato plants and allowed to produce additional F_1 progeny. This allowed us to test F_1 progeny synchronously with the F_2 and backcross populations. The adapted parental population (UMT) used in these crosses had been fed only tomato as larvae for four generations, although adults of each generation had been maintained on potato. The unadapted parental population (NYP) had not been previously exposed to tomato and was used as a control. To evaluate progeny from each cross, we caged 50 neonates in groups of 10 on five tomato plants (10 larvae per cage) for four days and repeated the test six times over time. The number and individual weight of survivors in each cage were recorded. We calculated mean larval weight and the CV of larval weights for each cage. Thus, for CV, larval survival, and mean larval weight, this was a randomized complete block design of nine populations (1 control, 2 F_1, 2 F_2, and four backcrosses), six replications, and five cages per replication. We subjected the proportions of the surviving larvae to an arcsine of square-root transformation. The CV's and mean larval weights were subjected to square-root transformation. We conducted 2-way ANOVAs and compared differences in CV, larval survival, and mean larval weight among populations as in 1992.

Hybridization in September 1993

In the previous experiment, we were unable to include one of the parental populations in our evaluation of larval performance, and a number of parents of the F_1 offspring died prematurely following their removal from cold storage. Therefore, during September 1993, we repeated the experiment with three modifications: F_2 generations were not tested, both parents were tested, and F_1 adults were not held in cold storage. In order to test all populations simultaneously, we regenerated the F_1 populations at the time that the backcrosses were made. The adapted parental population (UMT) in these crosses had been confined solely on tomato for 15 generations, and the unadapted parental population (NYP) in these crosses had no prior exposure to tomato. The experiment included six populations, five replications, and five cages per replicate. ANOVAs and data transformations for larval survival, CV of larval weight, and mean larval weight were conducted as in May 1993.

To gain more power in the statistical analyses of larval survival and larval weight, we combined data from experiments in May and September 1993. Each data set was treated as one block. F_2 data from the May block and UMT data from the September block were excluded from the analyses because neither of these populations were included in both blocks. For larval survival, CV of larval weight, and mean larval weight, the experiment included seven populations, six replications in May or five in September, two blocks, and five cages per replicate, with effects of replication nested within blocks. We used split plot ANOVAs to test differences among populations (same transformations as above). Because variances due to the effect of population by block were not significant for CV of larval weight and mean larval weight, we pooled these variances with the experimentwise error variances to gain additional degrees of freedom. All error terms used to test the effects of population, block, and replication were generated by RANDOM statements (PROC GLM, SAS Institute, 1989), using block, replication nested within blocks, population by block (if applicable), and population by replication nested within blocks as the random factors. We used Tukey's test to compare these differences among populations.

Test for sex linkage

From the hybridization experiments above, we observed variation between F_1 crosses and among back-

crosses in both larval survival and larval weight that was not fully explainable on the basis of autosomal inheritance or maternal cytoplasmic effects. We, therefore, hypothesized a sex-linked model; in which the sex chromosomal genotypes were *RR* for adapted UMT female, *RO* for adapted UMT male, *ss* for unadapted NYP female, *sO* for unadapted NYP male, with allele *R* dominant over *s*. F_1 progeny from the UMT $*$ NYP cross would be represented by sex chromosome *Rs* females and *RO* males, whereas progeny from the NYP $*$ UMT cross would be represented by Rs females and *sO* males. Assuming random mating and 1:1 sex ratio under this hypothesis, there should be no differences in larval survival and larval weight of females between the reciprocal crosses, but survival and weight should be lower in males from crosses involving unadapted female parents (NYP $*$ UMT).

The parents used to generate reciprocal F_1 crosses to test this hypothesis were UMT, which had been selected on tomato as larvae, and NYP, from a colony maintained exclusively on potato. We used 100 neonates from each parental (UMT and NYP) and F_1 population (UMP $*$ NYP and NYP $*$ UMP). These were confined in groups of 10 on 10 tomato plants as described previously. We conducted eight replicates of this experiment. Thus, the experiment involved four populations, eight replications, and 10 cages, and the total number of larvae tested from each population was 800 (400 females & 400 males, assuming a sex ratio 1:1). We recorded larval survival for each cage and measured individual weights of the survivors at the end of four days. Survivors were raised to adult (see Figure 6a) so their gender could be determined. To minimize further effects of selection for adaptation to tomato, each survivor was reared individually on potato foliage in petri dishes containing a strip of moist paper towel (27 °C and a photoperiod of 14L:10D). Foliage was replaced and mortality recorded daily. When the larvae reached the prepupal stage, they were transferred to plastic cups containing soil as a pupation medium. Once an adult emerged, its sex was recorded.

We used a G- test (Sokal & Rohlf, 1969) to test whether the sex ratio of adults differed between the reciprocal F_1 crosses. Two-way ANOVAs were conducted on 4-day larval survival and survival to adult (arcsine of square-root), and on mean larval weight per cage (log). Comparisons of differences among populations were the same as in 1993. In addition, we tested whether the sex by population interaction had a significant effect on larval weight. If males of genotype *RO* grew faster than males of genotype *sO*, we would expect a significant sex by population interaction or a difference in larval weight between the males of the reciprocal F_1 crosses. Because of death of larvae before adult emergence (see Figure 6 for survival to adulthood), we were able to use only a portion of the original data set of larval weight; consequently observations within a cage were highly unbalanced. We sorted the data by sex, population, and replication, and calculated mean larval weight across cages for each replication. The resulting experimental design included four populations, two sexes, and eight replications. We used a split plot ANOVA on the data of mean larval weight per replication (log transformation). Because the variances due to sex by population interaction were not significant, we pooled them with the experimentwise error variances. Effects of population were tested against a population by replication interaction. All other effects were tested against the pooled experimentwise error.

To gain additional statistical power to detect the differences in larval survival and larval weight between the reciprocal F_1 crosses, we combined only the F_1 data from this experiment with data from the May 1992 and May and September 1993 experiments. Each of these experiments were treated as blocks in the analysis. Thus, the experimental design involved two populations (UM $*$ NY & NY $*$ UM), four blocks, 5–8 replications, and 5–10 cages, with effects of replication nested within blocks. We used split plot ANOVAs to test differences in larval survival (arcsine of square root) and mean larval weight (log). All error terms used to test the effects of population, block, and replication were generated by RANDOM statements (PROC GLM, SAS Institute, 1989), using block, replication nested within blocks, and population by replication nested within blocks as the random factors. We used Tukey's test to compare these differences between the reciprocal F_1 crosses using effects of population by replication nested within blocks as the error term.

Results

Hybridization in May 1992

F_1 cross. Analyses of the 1992 data revealed significant differences in larval survival among populations ($F_{3,18} = 5.81$; $P = 0.0059$) and replications ($F_{6,18} = 2.66$; $P = 0.0502$), as well as a significant interaction between population and replication ($F_{18,252} = 3.96$;

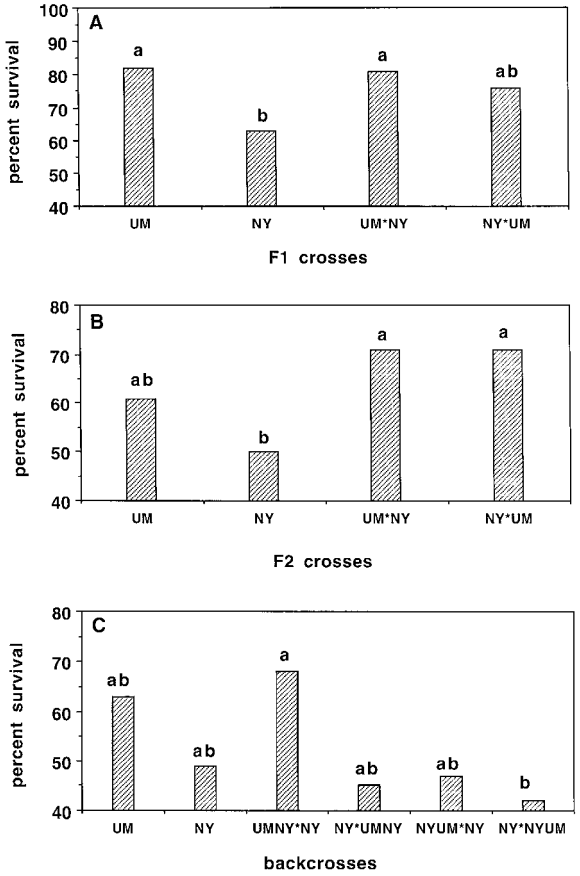

Figure 1. Larval survival (% ± SE) of F_1, F_2, and backcross populations after four days on tomato in May 1992 (a, b, and c, respectively). Means with the same letters are not significantly different at $\alpha = 0.05$ by Tukey's studentized range test.

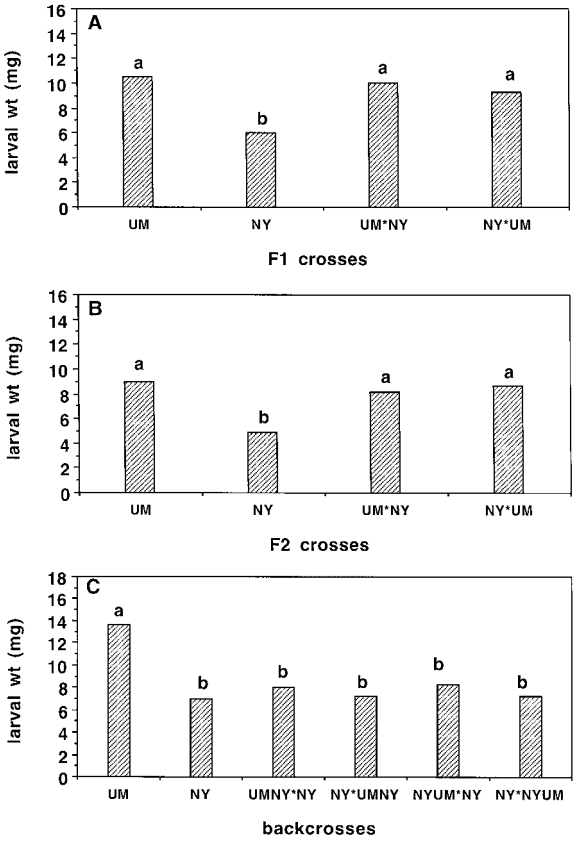

Figure 2. Mean larval weights (mg ± SE) of F_1, F_2, and backcross populations after four days on tomato in May 1992 (a, b, and c, respectively). Means with the same letters are not significantly different at $\alpha = 0.05$ by Tukey's studentized range test.

$P = 0.0001$). Larval survival of the reciprocal F_1 populations on tomato did not differ from each other or from the UM parent; survival of the UM ∗ NY population and the UM parent was significantly higher than the NY parent (Figure 1a), suggesting dominance derived from alleles of the UM population and no significant maternal or cytoplasmic effects.

There were also significant differences in mean larval weight among populations ($F_{3,18} = 10.19$; $P = 0.0004$) and replications ($F_{6,18} = 4.76$; $P = 0.0045$), and a significant interaction between population and replication ($F_{18,252} = 6.54$; $P = 0.0001$). Mean larval weights of the reciprocal F_1 populations on tomato did not differ from each other or from the UM parent, but were significantly heavier than the NY parent (Figure 2a), suggesting dominance derived from alleles of the UM population and no maternal or cytoplasmic effects.

F_2 cross. Both larval survival and weight differed significantly among populations (survival: $F_{3,18} = 4.81$; $P = 0.0124$; weight: $F_{3,18} = 10.38$; $P = 0.0003$) and among replications (survival: $F_{6,18} = 8.12$; $P = 0.0002$; weight: $F_{6,18} = 30.72$; $P = 0.0001$). The population by replication interaction was also significant for both survival ($F_{18,253} = 4.01$; $P = 0.0001$) and weight ($F_{18,251} = 3.53$; $P = 0.0001$). Neither larval survival nor larval weights of F_2 populations from reciprocal crosses differed significantly from each other or from the UM parent, but both survival and larval weights were significantly greater in the F_2 populations than in the NY parent population (Figures 1b and 2b).

If one or a few loci coded for differences in larval weight, the CV for larval weight of the F_2 generation would be greater than that of the parental populations. When the population effect was tested using mean squares from the experimentwise error, there was no

significant difference ($F_{3,244} = 0.47$; $P = 0.7019$) among the parental and F_2 populations in CV for larval weight (mean across cages ±SE: UM parent = 0.5258 ± 0.0293, NY parent = 0.5499 ± 0.0235, F_2 (UM ∗ NY) = 0.5312 ± 0.0217, and F_2 (NY ∗ UM) = 0.5093 ± 0.0195, respectively), suggesting no apparent segregation in the F_2 generation. There were significant effects of replication ($F_{6,244} = 6.45$; $P = 0.0001$) and the population by replication interaction ($F_{18,244} = 1.81$; $P = 0.0247$) in CV for larval weight.

Backcross of F_1 to NY parents. Larval survival differed significantly among F_1 backcross populations ($F_{5,29} = 3.35$; $P = 0.0164$) and among replications ($F_{7,29} = 3.19$; $P = 0.0125$). There was also a significant population by replication interaction ($F_{29,371} = 7.17$; $P = 0.0001$). A Tukey's test revealed no significant differences in larval survival between the parents, but indicated that larval survival of the UMNY ∗ NY backcross, which had cytoplasm derived from the UM parent, was significantly greater than that of the NYUM ∗ NY backcross (Figure 1c).

Mean larval weight differed significantly among populations ($F_{5,29} = 7.28$; $P = 0.0002$) and among replications ($F_{7,29} = 10.04$; $P = 0.0001$). The population by replication interaction was also significant ($F_{29,365} = 5.76$; $P = 0.0001$). None of the backcross populations differed from the NY parent in mean larval weight, but all had significantly lower mean weights than the UM parent (Figure 2c). This provided no evidence for maternal or cytoplasmic effects on mean larval weight when reared on tomato.

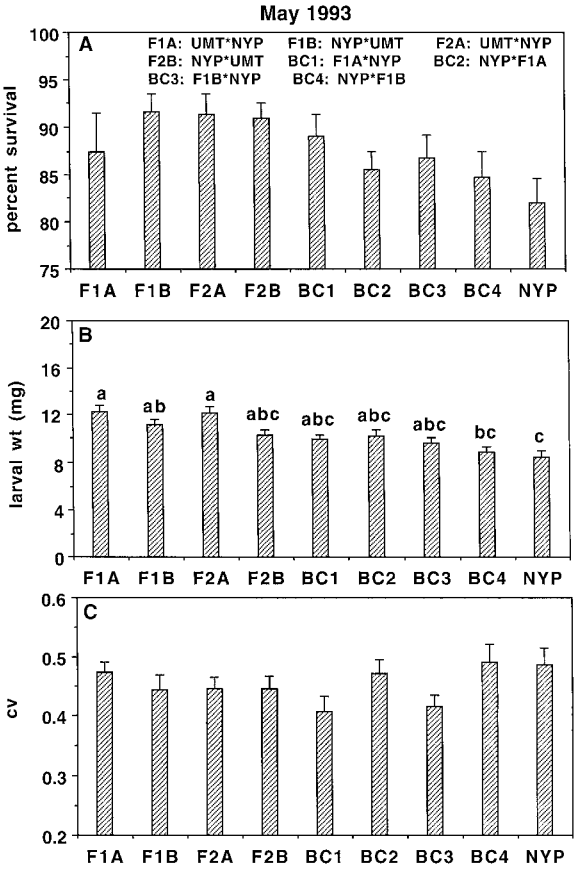

Figure 3. Larval survival (a), larval weight (b), and coefficients of variation (CV) for larval weight (c) of F_1, F_2, backcross, and parental NYP populations after four days on tomato in May 1993. Means (± SE) with the same letters are not significantly different at $\alpha = 0.05$ by Tukey's studentized range test. Effects of population on survival and CV were not significant in ANOVA at $\alpha \leq 0.05$.

Hybridization in May 1993

Larval survival did not differ significantly among populations ($F_{8,40} = 1.87$; $P = 0.0928$), but differed significantly among replications ($F_{5,40} = 3.43$; $P = 0.0113$) and the population by replication interaction was significant ($F_{40,211} = 1.53$; $P = 0.0301$). The absence of a significant difference in larval survival between the reciprocal F_1 populations (Figure 3a) indicates that there were no maternal or cytoplasmic effects on survival (note that we did not have data on the parental UMT population in this experiment). Survival of progeny from the backcross UMNY ∗ NY (BC1 in Figure 3a) tended to be the highest among the backcrosses, as was the case in 1992 (Figure 1c).

The effects of population ($F_{8,40} = 4.74$; $P = 0.0004$) and replication ($F_{5,40} = 3.57$; $P = 0.0092$) on mean larval weight were significant, as was the population by replication interaction effect ($F_{40,210} = 1.61$; $P = 0.0170$). There were no significant differences in mean larval weight between the reciprocal F_1 populations or among backcross populations (Figure 3b). Thus, there was no evidence for maternal cytoplasmic effects on the difference in mean larval weight between the parental UMT and NYP populations. The F_2 cross between UMT females and NYP males (UMT ∗ NYP), which had UMT cytoplasm, and both reciprocal F_1 crosses were significantly heavier than the NYP parent (Figure 3b).

There were no significant effects of population (F_1, F_2, backcross, and parental) ($F_{8,210} = 1.74$; $P = 0.0917$), replication ($F_{5,210} = 1.86$; $P = 0.1024$), or population by replication interaction ($F_{40,210} = 1.34$; $P = 0.0964$) on CV for larval weight (square-root transformation). This indicates that variance in larval

weight was not greater in the F_2 populations than in the F_1 populations, and suggests that there was no segregation of major genes for adaptation to tomato in the F_2 populations (Figure 3c).

Hybridization in September 1993

Larval survival (arcsine of square-root transformation) differed significantly among populations ($F_{7,28} = 4.37$; $P = 0.0022$), but not among replications ($F_{4,28} = 1.06$; $P = 0.3946$). The population by replication interaction was significant ($F_{28,160} = 1.84$; $P = 0.0106$). Larval survival of the F_1 population UMT $*$ NYP was significantly greater than survival of the backcross populations (NYP $*$ UMTNYP) and NYP $*$ NYPUMT) (Figure 4a). Survival of the backcross population UMTNYP $*$ NYP tended to be numerically higher than the other backcrosses, as was the case in the May 1992 and 1993 experiments. Although survival did not differ significantly between the parental populations, it tended to be greater in the UMT (87%) than the NYP (79%) populations (Figure 4a). As in previous experiments, survival did not differ significantly between the reciprocal populations, and was numerically at least as high as that of the adapted UMT parent population, again suggesting dominance but no maternal cytoplasmic effects (Figure 4a).

Analyses of mean larval weights revealed significant population ($F_{7,28} = 7.70$; $P = 0.0001$) and replication ($F_{4,28} = 28.10$; $P = 0.0001$) effects, as well as a significant population by replication interaction ($F_{28,160} = 2.84$; $P = 0.0001$). Larvae of the UMT parental population were significantly heavier than those of the NYP population (Figure 4b). Larvae of the reciprocal F_1 populations did not differ in mean weight from the UMT parent population, but were significantly heavier than larvae of the NYP parent population, again suggesting dominance effects derived from alleles of the UMT parent (Figure 4b). Mean larval weight did not differ significantly between the reciprocal F_1 populations (Figure 4b). Although there were no significant differences among the backcrosses (Figure 4b), mean larval weight of the NYP $*$ NYPUMT) backcross (BC4), which had the least cytoplasm derived from the UMT parental population, was significantly lower than that of the UMT parent (Figure 4b). Larvae of the UMTNYP $*$ NYP backcross (BC1), which had the most cytoplasm derived from the UMT parent, were significantly heavier than larvae of the NYP parent (Figure 4b). These last two results provide some indication that maternal cytoplasmic effects might affect the larval weight on tomato of progeny from crosses between the UMT and NYP populations.

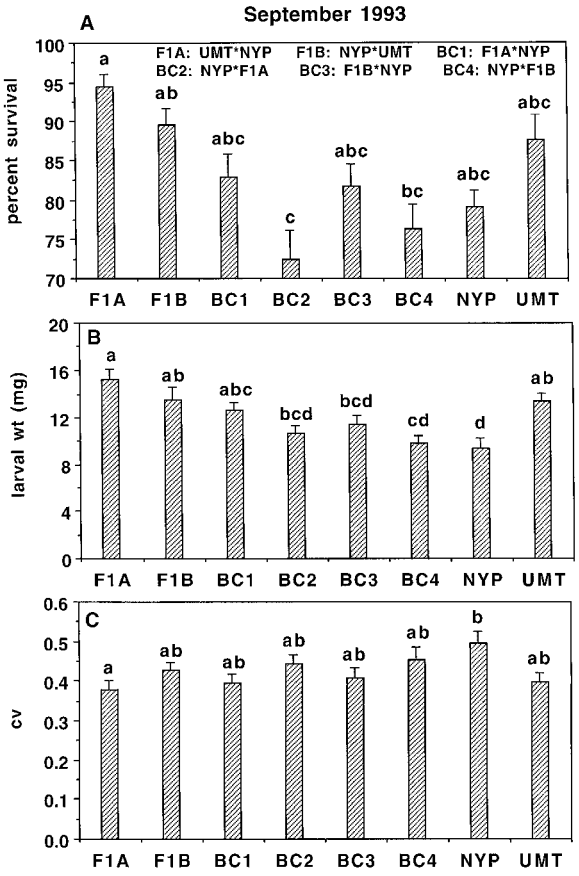

Figure 4. Larval survival (a), larval weight (b), and coefficients of variation (CV) for larval weight (c) of F_1, backcross, and parental populations after four days on tomato in September 1993. Means ($\pm$ SE) with the same letters are not significantly different at $\alpha = 0.05$ by Tukey's studentized range test.

The CV for larval weight was significantly affected by population ($F_{7,160} = 2.36$; $P = 0.0257$) but not by replication ($F_{4,160} = 1.34$; $P = 0.2583$) or the population by replication interaction ($F_{28,160} = 0.88$; $P = 0.6387$). The effect of population resulted because the CV for larval weight was significantly greater for the NYP population than for the UMT $*$ NYP population. No other differences between populations were significant (Figure 4c). These results suggest that differences in growth of the UMT and NYP populations on tomato are not controlled by only a few major genes inherited in a Mendelian fashion.

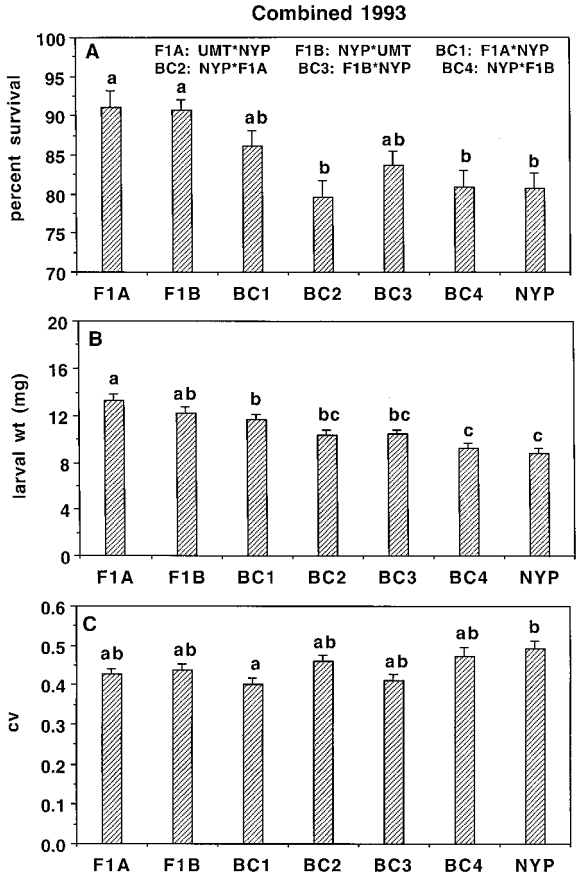

Figure 5. Larval survival (a), larval weight (b), and coefficients of variation (CV) for larval weight (c) of F_1, backcross, and both parental populations after four days on tomato when combining data from May and September 1993. Means ($\pm$ SE) with the same letters are not significantly different at $\alpha = 0.05$ by Tukey's studentized range test.

When the data sets from the May and September 1993 experiments were combined, the differences among populations in larval survival were not significant, but differences in mean larval weights were significant (Table 1). The reciprocal F_1 populations did not differ in mean larval weight, but larvae of the F_1 population UMT $*$ NYP were significantly heavier than larvae of all four backcross populations. However, larvae of the reciprocal F_1 population, NYP $*$ UMT, were significantly heavier than larvae of the backcross population NYP $*$ NYPUMP (BC4) (Figure 5b). Larvae of the NYP parental population were significantly lighter than larvae of the reciprocal F_1 populations and the backcross population UMT-NYP $*$ NYP (BC1), but did not differ in weight from any of the other backcrosses (Figure 5b). In addition, larvae of the backcross UMTNYP $*$ NYP (BC1) were significantly heavier than backcross NYP $*$ NYPUMT (BC4) (Figure 5b).

Analysis of combined data from the May and September 1993 experiments indicated that the CV of larval weight differed significantly between the UMT-NYP $*$ NYP backcross (BC1) and the NY parent populations , but not between any of the other populations (Table 1; Figure 5c).

Reciprocal F_1 test for sex linkage

The effects on larval survival at four days of population ($F_{3,21} = 6.37$; $P = 0.0031$), replication ($F_{2,287} = 54.88$; $P = 0.0001$), and the population by replication interaction ($F_{21,287} = 2.60$; $P = 0.0002$) were significant. The effects on survival to adult of population ($F_{3,21} = 12.39$; $P = 0.0001$), replication ($F_{3,287} = 31.65$; $P = 0.0001$), and the population by replication interaction ($F_{21,287} = 2.40$; $P = 0.0007$) were also significant. Neither larval survival at four days nor survival to adult differed significantly between the UMT population and the reciprocal F_1 populations, or between the reciprocal F_1 populations (Figure 6a). Both survival parameters were significantly lower for the NYP population than for the UMT and the reciprocal F_1 populations (Figure 6a). The sex ratio was homogeneous among populations ($G_{h,4} = 1.14$, $P = 0.7661$) and did not differ from 1:1 (female : male = 621 : 598, $G_{p,1} = 0.51$, $P = 0.434$). These results provide no support for the hypothesis that larval survival on tomato is conditioned by sex linked traits.

Four day mean larval weights were affected by population ($F_{3,21} = 12.01$; $P = 0.0001$), replication ($F_{7,287} = 42.59$; $P = 0.0001$), and the population by replication interaction ($F_{21,287} = 3.20$; $P = 0.0001$). The sex by population interaction was not significant ($F_{3,28} = 0.26$, $P = 0.8533$), indicating that the relative weights of males and females in the reciprocal F_1 and parent populations did not differ (Figure 6c). As in the previous experiments, larvae from the NYP population weighed significantly less after four days on tomato than larvae of the UMT and reciprocal F_1 populations (Figure 6b).

When averaged across populations, the four day mean larval weight of females was significantly higher than that of males (mean $\pm$ SE: 12.59 ± 0.69 & 11.89 ± 0.62, respectively; $F_{1,28} = 5.59$; $P = 0.0252$; log transformation). Mean larval weight averaged across sexes differed significantly among populations ($F_{3,21} = 4.94$; $P = 0.0094$; error term = population by replication interaction), but the sex by popula-

Table 1. ANOVAs for coefficient of variation in larval weight (square root transformation), larval survival (arcsine of square root transformation) and mean larval weight (square root or log transformation, respectively) when combined data from (1) May and September of 1993 and (2) May 1992, May and September of 1993, and May 1994. Error terms for each variance source were generated by RANDOM statements using PROC GLM (SAS Institute, 1989)

Source	CV				Larval survival				Mean larval weight			
	df	MS	F	P	df	MS	F	P	df	MS	F	P
1												
Block	1	0.0386	5.14	0.0497	1	0.3630	3.01	0.1213	1	6.4152	2.01	0.1900
Population	6	0.0319	3.02	0.0118	6	0.2395	3.64	0.0709	6	2.9126	12.22	0.0001
Population ∗ block	–	–	–	–	6	0.0658	1.52	0.1906	–	–	–	–
Replication (block)	9	0.0075	0.71	0.6957	9	0.0982	2.27	0.0309	9	3.1849	13.37	0.0001
Population ∗ replication (block)	60	0.0105	1.15	0.2281	54	0.0435	1.68	0.0039	60	0.2393	1.84	0.0005
Error	302	0.0092			303	0.0260			302	0.1303		
2												
Block					3	0.9769	1.83	0.1708	3	1.5644	1.99	0.1431
Population					1	0.0002	0.00	0.9504	1	0.8157	5.71	0.0243
Population ∗ block					3	0.0997	2.02	0.1346	3	0.0589	0.38	0.7676
Replication (block)					22	0.6020	11.98	0.0001	22	0.8841	5.25	0.0001
Population ∗ replication (block)					22	0.0502	1.16	0.2841	22	0.1685	3.01	0.0001
Error					352	0.0434			351	0.0559		

tion interaction was not significant ($F_{3,28} = 0.26$, $P = 0.8533$); thus the relative weights of males and females did not differ between the reciprocal F_1 populations (Figure 6c). As was the case with survival, these results provide no support for the hypothesis that larval weight gain on tomato is conditioned by sex linked traits.

Analyses of the combined data from this experiment and the May 1992 and May and September 1993 experiments did not detect a significant difference in larval survival between the reciprocal F_1 populations (UM ∗ NY = 81.30% ± 1.34; NY ∗ UM = 82.15% ± 1.20; Table 1), suggesting that maternal effects were not important. However, mean larval weight of the UM ∗ NY population (12.29 ± 0.31 mg) was greater than that of the NY ∗ UM population (11.23±0.29 mg) (Table 1), suggesting that maternal effects might contribute to the difference in larval weight between the adapted UM and unadapted NY population.

Discussion

In a previous study involving both the UM and NY populations used in the present study, Lu et al. (1997) documented the existence of genetic variation in four day larval survival and weight on tomato, as well as the ability of both populations to respond to selection for adaptation to tomato. Our analyses of four day larval survival and weight on tomato in reciprocal F_1, F_2, and backcross populations indicate that the genetic mechanisms underlying differences in adaptation of these UM and NY populations are complex.

Results of F_1 crosses conducted by Lu et al. (1997) using these populations indicated that adaptation to tomato was a dominant trait that had no maternal or cytoplasmic components. However, F_1 crosses cannot clearly distinguish the effects of adaptive, dominant genes from the effects of heterosis (hybrid vigor). We conducted F_2 crosses because heterotic effects usually begin to break down in the F_2 generation. Furthermore, if only one or a few dominant alleles were involved in adaptation to tomato, we expected the F_2 offspring to display extra variation in growth rate due to segregational variation.

If growth on tomato involved a large number of dominant alleles at many loci, we would not expect to see a large increase in CV of weights in the F_2. However, we would expect to observe a decrease in the mean weight of F_2 larvae compared to F_1 larvae, given dominant control of the adaptive phenotype, because most F_2 larvae would lack adaptive alleles at some of the loci, whereas all of the F_1 larvae would have

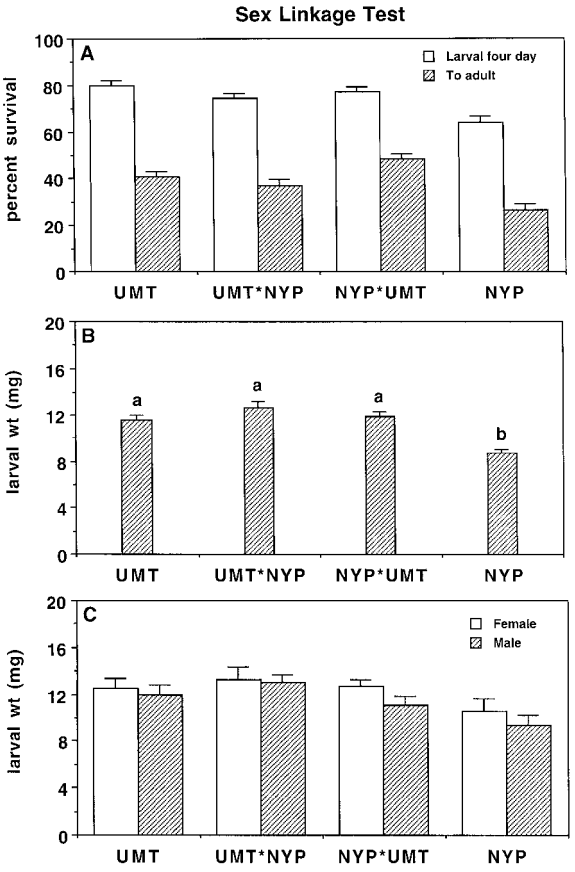

Figure 6. Larval survival to day four and survival to adult (a), four day larval weights (sexes not distinguished) (b), and four day larval weights of males and females that survived to become adult (c) for F_1 and both parental populations after four days on tomato in May 1994. Means ($\pm$ SE) with the same letters are not significantly different at $\alpha = 0.05$ for by Tukey's studentized range test.

one adaptive allele at each locus (assuming fixation in the parents). Backcrosses were also conducted because they would be more powerful for detecting some added segregational variation. Like the F_2 larvae, the mean weight of backcross larvae is expected to be lower than that of the F_1 larvae, if many dominant alleles were involved in weight gain.

Weight data from the F_2 tests were perplexing. Contrary to expectation, the F_2 larvae were as large as the UM larvae and the CV's of larval weight in the F_2 larvae were equal to or lower than the CV's for the parental strains and F_1 larvae. In the first backcross test, the backcross larvae were significantly smaller than those from the UM strain and were surprisingly similar in weight to those of the NY strain. The CV's for the backcrosses were no higher than those of the parental strains. In the September 1993 experiment, some of the backcrosses resulted in larger larvae than were produced by the NY parental strain. But here again, the CV's were never higher than those of the parental strains.

Examination of the first sets of crosses indicated that crosses involving UM females resulted in larger larvae than crosses involving NY females. We therefore designed additional crosses to determine if a cytoplasmic factor influenced weight gain or survival. Although the backcross in this experiment provided some evidence for a cytoplasmic factor, the effect was small. A final experiment designed to determine if sex linkage of adaptive alleles could explain some of our initial F_2 results revealed no effects of sex linkage.

After conducting this elaborate set of genetic crosses, we remain unable to provide a classical genetic explanation of the finding that F_2 offspring are equal in size and variance to the offspring from the tomato adapted parent. We encountered considerable variation between experiments conducted at different times (especially in larval survival, e.g., larval survival in the May and September 1993 experiments), perhaps due to seasonal differences in light intensity despite supplemental illumination. We also repeatedly observed a significant population by replication interaction in our experiments. Nonetheless, the same general patterns of differences among populations occurred repeatedly and previous work has shown that these populations responded to selection on tomato (Lu et al., 1997). Consequently, we feel confident that the among population differences we examined have a significant genetic component. Although it is possible that our odd experimental results were due to some unique feature of our *L. decemlineata* strains and the chemistry of tomato, examination of previously published genetic studies of adaptation to various host plants by *L. decemlineata* reveals a pattern of odd results. Hsiao (1982) compared performance on potato of a *L. decemlineata* strain from Mexico with a laboratory strain that was adapted to potato. When reared on potato, the male and female pupae of the laboratory strain were approximately 1.5 times heavier than the pupae from the Mexican strain. Hsiao (1982) also examined pupal weights of F_1 and F_2 offspring from reciprocal crosses of these two strains when reared on potato. Hsiao's F_1 results indicated that the trait was partially to fully dominant with a potential maternal or sex linkage effect (F_1 pupae were larger if the female in the cross was from the laboratory strain). In crosses where the initial females were from the laboratory strain, the F_2 pupal weights were lower than the

F_1 pupal weights. When the initial females were from the Mexican strain, the F_2 pupal weights were slightly greater than were those of the F_1. The most perplexing result of Hsiao's study was that the CV's for the F_2's were smaller than the CV's for the F_1's.

Hare and Kennedy (1986) compared life history traits of a *L. decemlineata* strain from Connecticut (CT) and a strain from North Carolina (NC) when reared on *Solanum carolinense*. Overall, the NC strain was better adapted for growth on *S. carolinense* than the CT strain. All of their experiments were conducted in duplicate; one set was carried out in a CT laboratory and the other set was carried out in a NC laboratory. In the NC laboratory, survival of NC, CT, and F_1 larvae on *S. carolinense* averaged 31.9%, 2.5%, and 31.9%, respectively. In the Connecticut laboratory, survival of the NC, CT, and F_1 larvae averaged 45.6%, 13.1%, and 41.6%, respectively. Survival on *S. carolinense* was, therefore, mostly to completely dominant. Survival of F_2 larvae was 44.1% and 37.8%, respectively, in the NC and CT laboratories. Combining the results from both laboratories indicates that the F_1 and F_2 larvae had similar survival. This would not be expected if a few dominant genes were involved in adaptation to *S. carolinense*. Other results from the Hare & Kennedy (1986) study indicate that the means and CV's for adult weight in the F_1 and F_2 are similar, but detailed comparisons are not justified because of large standard errors.

Finally, Pelletier & Smilowitz (1991) reported the results of a quantitative genetics experiment to analyze genetic variation in adaptation to potato and *S. berthaultii* within a single population of *L. decemlineata*. Their results point to some interesting inconsistencies. For developmental time on *S. berthaultii* and potato, they observed a stronger effect of the male parent than the female parent, which resulted in a high estimate of additive genetic variance and what would be a negative effect of non-additive genetic effects combined with maternal effects (the authors assigned these a value of zero). On *S. berthaultii*, they also observed a large effect of the male parent (sire) and a maternal/non-additive effect of zero for pupal weight. On potato, there was a non-significant sire effect on pupal weight, but the maternal effect/non-additive effect accounted for a large proportion of the variation in pupal weight. Again, these are unexpected results.

Results of our experiments and those previously reported by others indicate that the genetic basis for adaptation to host plants by *L. decemlineata* may involve more than simple Mendelian traits or maternal inheritance. We feel that further work using the classical crossing designs presented here will not be fruitful in explaining the genetic basis for these adaptive traits. The most promising approach is likely to involve development of a genomic map of *L. decemlineata* and a search for linkage groups that are associated with adaptation to specific host plants. The molecular genetic aspects of such an approach are becoming less formidable as genomic technology becomes more eficient. The real challenge in using a QTL approach to address this plant/herbivore system is likely to reside in the high level of environmental variance associated with growth of larvae on their host plants. It should be possible to deal with seasonal variation in host plant quality by testing large numbers of F_2 and backcross offspring at the same time. However, our experiments typically found a population by replication interaction within each time period, so a very carefully designed experiment with large numbers of offspring would be needed in order to gain the statistical power needed to detect QTLs.

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