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Research article

Costs and stability of cabbage looper resistance to a nucleopolyhedrovirus

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Abstract. The goal of this study was to examine the possible costs and the stability of the resistance of cabbage loopers (*Trichoplusia ni*) to the single (S) nucleocapsid nucleopolyhedrovirus of *T. ni* (TnSNPV). Resistance to the virus did not appear to incur any measurable fitness costs under laboratory conditions. When reared in the absence of the virus, there was no difference in the number of eggs produced or egg hatch of control and selected individuals. There even was a tendency for selected cabbage loopers to develop faster and to produce heavier pupae. The difference in the pupal weight and developmental time of control and selected *T. ni* did not covary with the number of generations of selection. Furthermore, the offspring of hybrid crosses (control × selected moths) were as fit as those of pure pairings (control × control or selected × selected adults). Finally, the resistance of cabbage loopers to TnSNPV did not decline when exposure to the virus ceased for nine generations.

Key words: biological control, coevolution, fitness costs, geographic mosaic theory, nucleopolyhedrovirus, Red Queen Hypothesis, TnSNPV, *Trichoplusia ni*

Introduction

Hosts and pathogens have traditionally been viewed as being caught in a coevolutionary 'arms race' in which hosts become increasingly resistant and pathogens more and more virulent (Red Queen Hypothesis of Van Valen, 1973). However, recent theoretical models have challenged this view and questioned the ubiquity of such arms races (see Saloneimi, 1993; Abrams and Matsuda, 1996; Gavrilets, 1997). The models predict that an evolutionary Red Queen will emerge only when the evolution of host resistance and pathogen virulence are not constrained. If these conditions are not satisfied, the host and pathogen are expected to enter cyclical chase dynamics.

One factor that could affect the dynamics of host–pathogen coevolution is the cost hosts incur to become resistant (Saloneimi, 1993; Abrams and

Matsuda, 1996; Gavrilets, 1997; Brodie and Brodie, 1999). Resistance is frequently assumed to involve the diversion of energy from growth and/or reproduction to combat the pathogen, and thus resistant individuals could be less fit than susceptible ones when reared in the absence of the pathogen. Simms and Rausher (1987) proposed that these costs will play an important role in determining the level and stability of host resistance. If resistance does not incur any costs then selection should lead to fixation of the resistance allele(s) (Mode, 1958; Gillespie, 1975; Simms and Rausher, 1987). Alternatively, when resistance is costly, selection should favor an intermediate level of resistance and hosts should evolve so as to maximize the difference between the benefits and costs of resistance (Simms and Rausher, 1987). Also, when resistance is costly, it is expected to be unstable and to decline when exposure to the pathogen stops.

Costs of resistance are often viewed as being static and independent of the level of resistance. However, the costs could be dynamic and this could influence the evolutionary outcome of resistance. Prolonged exposure to a pathogen could lead to resistance coadaptation and the costs of resistance could decline as resistance evolves. Blowflies (*Lucilla cuprina*) that evolved resistance to the chemical diazinon, later evolved compensatory genetic changes that lessened the cost of resistance (McKenzie *et al*., 1982; McKenzie and Purvis, 1984). Alternatively, the cost of resistance could increase with the level of resistance. In their model, Boots and Haraguchi (1999) allowed resistance to be increasingly costly (convex trade-off) or for the costs to plateau at high levels of resistance (concave trade-off), and showed that the evolutionary outcome for resistance depended on the shape of the trade-off curve. Hence, monitoring costs as resistance evolves may provide an explanation for the evolutionary trajectory of resistance.

In the majority of studies, costs of resistance have been investigated by comparing the fitness of susceptible and resistant individuals reared in the absence of the pathogen and little attention has been given to hybrids (offspring of susceptible and resistant crosses). However, the potential costs incurred by hybrids could be critical in the development of resistance, particularly in the early stages when most resistance genes are likely to be found in hybrids (e.g. Georghiou, 1983).

The present study is part of our ongoing research on the coevolution of cabbage loopers (*Trichoplusia ni*) and their nucleopolyhedrovirus (the single (S) nucleocapsid nucleopolyhedrovirus of *T. ni*; TnSNPV). Cabbage loopers are cosmopolitan, highly destructive, herbivorous caterpillars that are known to feed on over 150 species of plants (Lindgren and Greene, 1984). TnSNPV is a lethal pathogen commonly found in wild *T. ni* populations (Lindgren and Greene, 1984). It also shows a great deal of potential for being used as a bioinsecticide against cabbage loopers (Jaques, 1977; Entwistle, 1983). Milks and Myers (2000) experimentally examined the coevolution of cabbage loopers and

TnSNPV, and observed that *T. ni* could evolve up to 22-fold resistance to the virus. However, they did not observe any change in the virulence of the virus. In this study, we examine the costs and stability of *T. ni* resistance to TnSNPV. Specifically, we compare the fitness of susceptible and resistant cabbage loopers reared in the absence of TnSNPV, and monitor costs as resistance evolves. We also investigate the costs of resistance incurred by hybrid *T. ni*, and examine if resistance to TnSNPV declines when exposure to the virus ceases.

Materials and methods

Establishment of selected lines (Milks and Myers, 2000)

Selection Experiment 1 consisted of two control (lines C1 and C2) and two selected (lines S1 and S2) lines of 500 cabbage loopers. Each line was initially established by taking 100 neonates from five *T. ni* populations (only those populations were available at that time). The larvae of lines S1 and S2 were infected with 11,000 occlusion bodies (OBs) of TnSNPV while those of lines C1 and C2 were treated with distilled water. For each selected line, the larvae that succumbed to TnSNPV were pooled, the virus was isolated from these cadavers and was used to infect the progeny of the survivors of that line. The offspring of the survivors of lines C1 and C2 were treated with distilled water. Selection was carried out for eight generations, discontinued between generations 9–14, and resumed from generations 15–19.

Experiment 2 was initiated at the end of Experiment 1 mainly to confirm the findings of the first experiment and consisted of one control (line C3) and one selected (line S3) line of 800 individuals established by taking 66 or 67 neonates from 12 *T. ni* populations. The same protocol as in Experiment 1 was used with the exception that the larvae of line S3 were infected with 14,000 OBs of TnSNPV, and selection was carried out for 26 generations without interruption. At the conclusion of Experiments 1 and 2, cabbage loopers had respectively evolved 5- (mean LD_{50} of lines S1 and S2 divided by the mean LD_{50} of lines C1 and C2) and 22-fold (LD_{50} of line S3 divided by the LD_{50} of line C3) resistance to TnSNPV. The virulence of the virus did not change in either experiment. See Milks and Myers (2000) for further details on the selection experiments.

Fitness in the absence of TnSNPV

Control and selected individuals

At various generations (G) (G1–10, 16 and 20 of Experiment 1; G4, 5, 13–17, 24 and 27 of Experiment 2) eggs from control and selected lines were surface-

sterilized by soaking them in 1.5% sodium hypochlorite, hatched and 40–50 neonates per line were reared individually in 30-ml plastic cups containing high wheat germ diet at 26 ± 1 °C with a photoperiod of 16:8 (L:D) (see Milks, 1997). Insects were checked daily and pupal weight (on the day of pupation), sex and developmental time to adult emergence were recorded (pupal weight and developmental time were not recorded at G13, 15, 17 and 24 of Experiment 2). For Experiment 1, the average pupal weight (and developmental time) of male and female cabbage loopers of each line were calculated, and the means compared using ANOVA (PROC GLIM: SAS Institute, 1990) with treatment (control or selected), sex and treatment × sex as factors. Unfortunately, in the case of Experiment 2, there was only one line per treatment (control line 3 and selected line 3) and thus to perform an ANOVA, each individual had to be considered as a separate datum. This approach may inflate the degrees of freedom and could increase the probability of detecting a spurious, significant cost of resistance. However, this does not appear to have been the case since the treatment factor in the ANOVA of pupal weight and developmental time was significant almost equally frequently in Experiment 1 (7/24; 29%) and Experiment 2 (3/10; 30%) (Table 1).

At G13–17 and 24 of Experiment 2, 8–10 pairs of adults of lines C3 and S3 were randomly chosen and placed in 500-ml paper cups (see Milks, 1997). Eggs were collected 4 days after a pair was formed and every second day for the next 6 days (G16) or until the female died (G13–15, 17 and 24). Also, at G16 and 24, eggs were incubated at 26 ± 1 °C and the hatching success was recorded. Again, since there was only one line per treatment in Experiment 2, each moth pair was considered as a datum. The mean number of eggs laid and the % hatching success (arcsin square root transformed) of control and selected pairs were compared using *T*-tests.

Hybrids

At G16 and 24 of Experiment 2, we established 10 pure (C3-F × C3-M, S3-F × S3-M; F = female, M = male; same pairs as those used in the previous section) and hybrid pairings of each type (C3-F × S3-M, S3-F × C3-M). The number of eggs laid per pair was counted at both generations and hatching success of eggs from pure pairings was also estimated at G16 and 24 (see previous section). However, the hatching success of eggs from hybrid pairings was only estimated at G24. Furthermore, 20 offspring from each pair of adults from G24 were reared individually and their pupal weight, developmental time and sex were recorded. Each pair was considered a separate datum. Pairing type (four levels) was the only factor in the ANOVA of egg production and % egg hatch (arcsin square root transformed) while pairing type, sex and sex × pairing type were

Table 1. *F*-values from two-way ANOVAs of pupal weight and developmental time to adulthood of cabbage loopers with sex ($df = 1$), treatment (control or selected for resistance to TnSNPV: $df = 1$) and the sex × treatment interaction term ($df = 1$) as factors ($n = 8$ for each generation of Experiment 1; $n = 78$ to 92 for Experiment 2)

Generation	Pupal weight			Developmental time		
	Sex	Treatment	Sex × Treatment	Sex	Treatment	Sex × Treatment
Experiment 1						
1	13*	0.3	0.7	3.6	2	<0.1
2	11*	4.9	0.4	3.3	3.3	1.3
3	34**	37**	8.2*	8.6*	1.2	<0.1
4	3.9	10*	0.2	11*	<0.1	<0.1
5	20**	2.3	0.2	9.4*	2.3	1.8
6	21**	14*	1	7.3*	<0.1	<0.1
7	7.7*	5.8*	0.1	9.2*	4.2	0.1
8	3.3	<0.1	0.3	15*	199**	4.6
9	3.4	<0.1	2.8	3.7	2.1	<0.1
10	17*	23*	0.1	12.6*	34**	<0.1
16	0.3	0.1	0.6	0.1	0.1	1.6
20	8.9*	2.6	0.3	8.9*	1.6	0.2
Experiment 2						
4	4.9*	0.1	0.6	12.4**	10.3**	5.3*
5	2	4.9*	0.1	2.5	0.2	0.7
14	12.5**	0.6	2	7.4**	0.4	1.9
16	6.4*	0.3	0.1	18.1**	0.1	0.1
27	0.1	0.1	1.7	0.3	22.2**	1.1

* $p < 0.05$; ** $p < 0.01$.

used in the ANOVA of the % survival to adulthood, and mean pupal weight and developmental time of the offspring.

Stability of resistance to TnSNPV when exposure to the virus stops

At G17 of Experiment 2, line S3 was randomly divided into two sublines of 800 individuals. In one subline (S3/TnSNPV+), selection for resistance to TnSNPV continued. In the other subline (S3/TnSNPV−), exposure to the virus stopped and the larvae were treated with distilled water for nine generations. At G27, 30 larvae (three replicates of 10) of line C3 were infected with 1500, 7500, 15,000 or 35,000 OBs of TnSNPV and 30 larvae (three replicates of 10) of subline S3/TnSNPV+ and subline S3/TnSNPV− were infected with 7500, 15,000, 35,000, 45,000 or 90,000 OBs of TnSNPV. Cohorts of 25 larvae per line were mock infected with distilled water. The LD_{50} of line C3 and subline S3/TnSNPV+ and subline S3/TnSNPV− were calculated using PROC PROBIT (SAS Institute, 1990: data pooled across replicates and not corrected for

control mortality since ≤5% of mock infected insects died) and were judged to be significantly different when their 95% CI did not overlap (e.g., Tabashnik *et al*., 1994). The slopes and intercepts of the dosage–mortality curves of line C3 and subline S3/TnSNPV+ and subline S3/TnSNPV− were also compared as described in Collett (1991).

Results

Fitness in the absence of TnSNPV

Control and selected individuals

The pupal weight and developmental time of control and selected *T. ni* reared in the absence of TnSNPV typically differed by <10% in both experiments (Figs. 1 and 2). In 8 of 10 generations in which the difference was significant, cabbage loopers that were selected for resistance produced heavier pupae and/or developed faster than those of the control lines (Table 1, Figs. 1 and 2). As expected, the main effect of sex was significant in many ANOVA of pupal weight and developmental time (Table 1). However, the sex × treatment term was seldom significant for either trait (Table 1), indicating that the treatment affected the pupal weight and developmental time of both sexes in the same manner. The % difference in the mean pupal weight of control and selected *T. ni* was not correlated to the number of generations of selection for either sex in Experiment 1 (females: Spearman $r = 0.11$; males: Spearman $r = -0.14$; both $n = 12$ and $p > 0.25$; Fig. 1A) or in Experiment 2 (females: Spearman $r = 0.30$, $p > 0.25$; males: Spearman $r = -0.66$, $p > 0.05$; both $n = 5$; Fig. 1B). There also was no relationship between the % difference in the mean developmental time of control and selected cabbage loopers and the number of generations of selection for either sex in Experiment 1 (females: Spearman $r = -0.08$; males: Spearman $r = -0.06$; both $n = 12$ and $p > 0.25$; Fig. 2A) or in Experiment 2 (females: Spearman $r = -0.30$; males: Spearman $r = 0.41$; both $n = 5$ and $P > 0.25$; Fig. 2B).

There also was no consistent difference in egg production of control and selected moths of Experiment 2. Control adults laid 25% more eggs at G24 whereas selected adults respectively produced 20 and 35% more eggs at G13 and 16. However, none of those differences were significant (*T*-tests, all $p > 0.07$). In the other generations (G14, 15 and 17), there was ≤10% difference in the egg production of control and selected pairs, and again none of the differences were significant (*T*-tests, all $p > 0.30$). The hatching success of eggs produced by control and selected pairs did not differ at G16 (mean ± SE: control: 79 ± 3%; selected: 81 ± 4%) and G24 (mean ± SE: control: 88 ± 4%; selected: 84 ± 2%; *T*-tests, both $p > 0.20$).

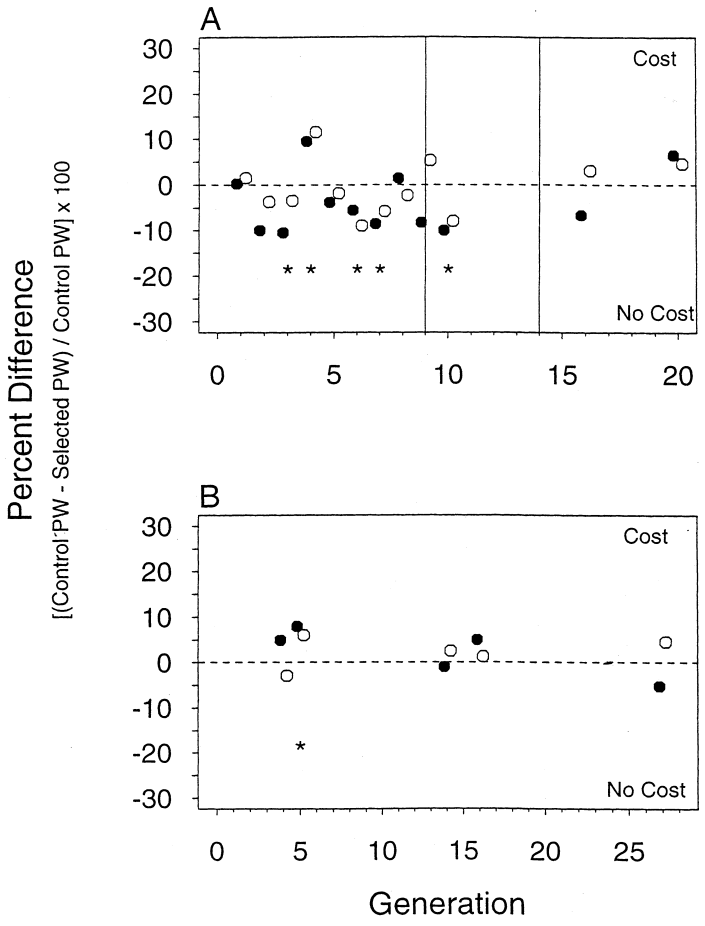

Figure 1. Percent difference in the mean pupal weight (PW) of control and selected lines. (a) Experiment 1 [(mean PW of control lines 1 and 2 − mean PW of selected lines 1 and 2)/mean PW of control lines 1 and 2] × 100. (b) Experiment 2 [(mean PW of control line 3 − mean PW of selected line 3)/mean PW of control line 3] × 100 (females ○, males ●). Percentages > 0 indicate that selected cabbage loopers produced lighter pupae than control insects i.e. resistance to TnSNPV was costly. There was no selection between generations 9–14 of Experiment 1. ∗ indicates that the effect of treatment (control or selected) was significant at $p = 0.05$ in the ANOVA of pupal weight (see Table 1).

Hybrids

The mean number of eggs laid per pair did not vary significantly among the four types of crosses at G16 ($F = 1.90$, $p = 0.15$) or G24 ($F = 0.8$, $p = 0.50$) of Experiment 2 (Fig. 3). There also was no difference in % egg hatch among crosses at G24 (mean ± SE: C3-F × C3-M: 88 ± 4%; C3-F × S3-M: 75 ± 3%; S3-F × C3-M: 89 ± 3%; S3-F × S3-M: 84 ± 2%; $F = 1.3$, $p = 0.30$). The survival of the offspring to adulthood in the absence of

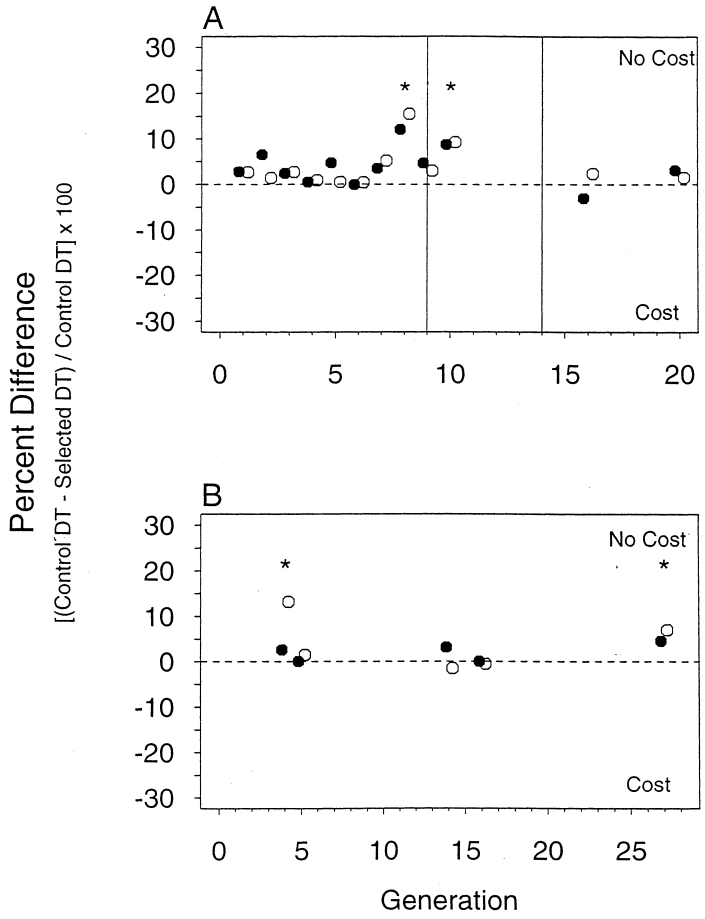

Figure 2. Percent difference in the mean developmental time (DT) of control and selected lines. (a) Experiment 1 [(mean DT of control lines 1 and 2 − mean DT of selected lines 1 and 2)/mean DT of control lines 1 and 2] × 100. (b) Experiment 2 [(mean DT of control line 3 − mean DT of selected line 3)/mean DT of control line 3] × 100 (females ○, males ●). Percentages < 0 indicate that selected cabbage loopers developed slower than control insects i.e. resistance to TnSNPV was costly. There was no selection between generations 9–14 of Experiment 1. ∗ indicates that the effect of treatment (control or selected) was significant at $p = 0.05$ in the ANOVA of developmental time (see Table 1).

TnSNPV did not vary among crosses (mean ± SE: C3-F × C3-M: 98 ± 1%; C3-F × S3-M: 95 ± 2%; S3-F × C3-M: 96 ± 2%; S3-F × S3-M: 97 ± 4%; $F = 1.3$, $p = 0.30$), and none of the individuals that died showed signs of nucleopolyhedrosis. The pupal weight of the offspring was independent of the type of the cross (Fig. 4A, Table 2). However, the developmental time of the offspring did vary among crosses (Fig. 4B, Table 2). Female offspring from S3-F × S3-M and S3-F × C3-M pairings developed faster than those from C3-F ×

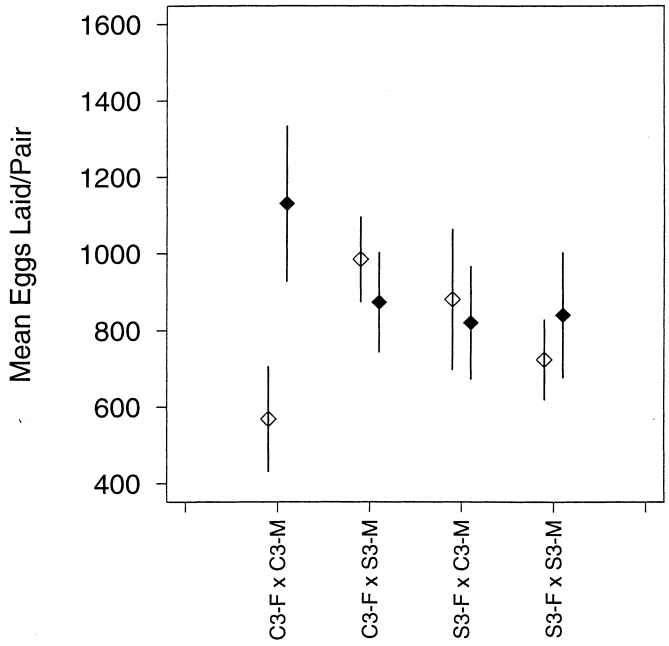

Figure 3. Mean (±1 SE) number of eggs laid per pair of adults from generation 16 (◇) and 24 (◆) of Experiment 2 in relation to the type of pairing. C3 = control line 3, S3 = selected line 3, F = female, M = male ($n = 10$ pairs per pairing type).

C3-M pairs (Tukey test: both $p < 0.02$; Fig. 4B). Male offspring from S3-F × S3-M pairs also developed faster than those from C3-M × C3-F pairs (Tukey test; $p < 0.05$; Fig. 4B).

Stability of resistance to TnSNPV when exposure to the virus stops

The survival of S3 cabbage loopers that continued to be exposed to TnSNPV between G19 and 26 (subline S3/TnSNPV+) varied between 35 and 85% (Fig. 5). During the same period, the survival of S3 caterpillars that were treated with water (S3/TnSNPV−) was >90%, and was nearly identical to that of line C3 (Fig. 5). The dosage–mortality curves of line C3, subline S3/TnSNPV+ and subline S3/TnSNPV− for G27 all had the same slope (Fig. 6; all $\chi^2 < 1.32$, $df = 1$, $p > 0.25$). Furthermore, the y-intercept of the dosage–mortality curves of subline S3/TnSNPV+ and subline S3/TnSNPV− did not differ (Fig. 6; $\chi^2 < 1.32$, $df = 1$, $p > 0.25$). However, they both differed from the y-intercept of the dosage–mortality curve of line C3 at G27 (Fig. 6; both $\chi^2 = 50$, $df = 1$, $p < 0.001$). Finally, the LD_{50} of sublines S3/TnSNPV+ and S3/TnSNPV− at G27 were not significantly different (S3/TnSNPV+: 109,248

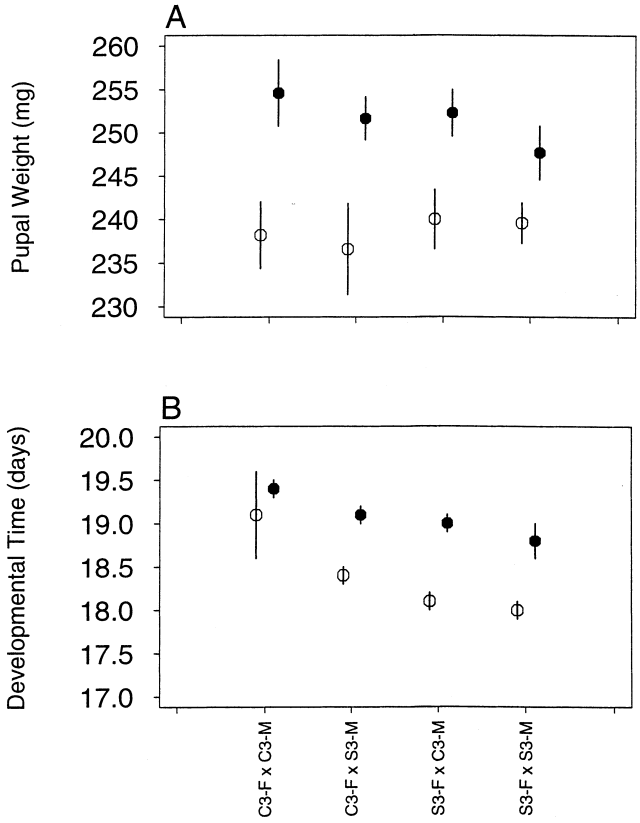

Figure 4. Mean (±1 SE) (a) pupal weight and (b) developmental time to adulthood of female (○) and male (●) offspring in relation to the type of pairing of their parents ($n = 10$ families per pairing type; each family consisted of 20 offspring per pair).

Table 2. *F*-values from two-way ANOVAs of pupal weight and developmental time to adulthood of the offspring with sex (*df* = 1), pairing type of the parents (C3-F × C3-M, C3-F × S3-M, S3-F × C3-M, S3-F × S3-M; C3 = control line 3, S3 = selected line 3; F = female, M = male; *df* = 3) and the sex × parental pairing type interaction term (*df* = 3) as factors. $n = 40$ families (four pairing types × 10 families per pairing type); each family consisted of 20 offspring

Factor	Pupal weight	Developmental time
Sex	26.9*	8.2*
Parental pairing type	0.4	7.1*
Sex × Parental pairing type	0.5	1.4

* $p < 0.01$.

OBs: 95% CI = 71,528–339,338; S3/TnSNPV−: 63,203 OBs: 95% CI = 37,995–185,882), but were both greater than that of line C3 (5056 OBs: 95% CI = 3309–7065).

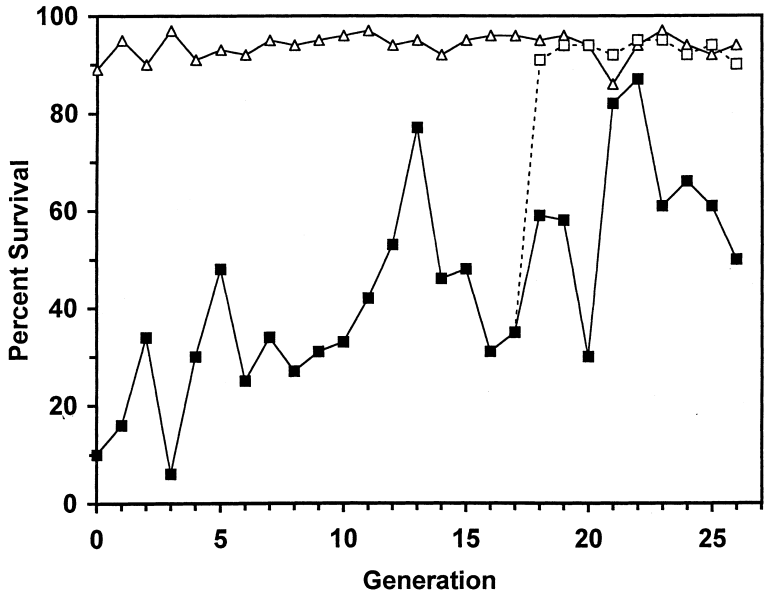

Figure 5. Percent survival of lines during selection. At each generation, caterpillars of line C3 (△) were mock infected with water. Larvae of line S3 were infected with 14,000 OBs of TnSNPV and, at generation 17, the line was randomly divided into two sublines: in one of them (subline S3/TnSNPV+; ■), selection for resistance to TnSNPV continued. In the other subline (subline S3/TnSNPV−; □), exposure to the virus stopped and larvae were treated with distilled water i.e. as those of line C3 ($n = 800$ individuals per line).

Discussion

Fitness in the absence of TnSNPV

Overall, there was little evidence that resistance to TnSNPV incurred fitness costs. In fact, there was a tendency for cabbage loopers that were selected for resistance to produce heavier pupae and to develop faster than control insects when reared in the absence of TnSNPV (Figs. 1, 2 and 4). Furthermore, hybrid individuals developed as fast and produced pupae that were as heavy as those of control individuals (Fig. 4). In general, there was no difference in the number of eggs laid and hatched by control, selected and hybrid pairs (Fig. 3). In contrast to our findings, resistance to NPVs or to granuloviruses adversely affected pupal weight, developmental time and/or reproductive success (egg production and hatch) in *Spodoptera frugiperda* (Fuxa *et al*., 1988), *Plodia interpunctella* (Boots and Begon, 1993) and *Anticarsia gemmatalis* (Fuxa and Richter, 1998).

It is unlikely that the lack of cost of resistance can be attributed to the duration of our selection experiment (15–26 generations) or to the level of

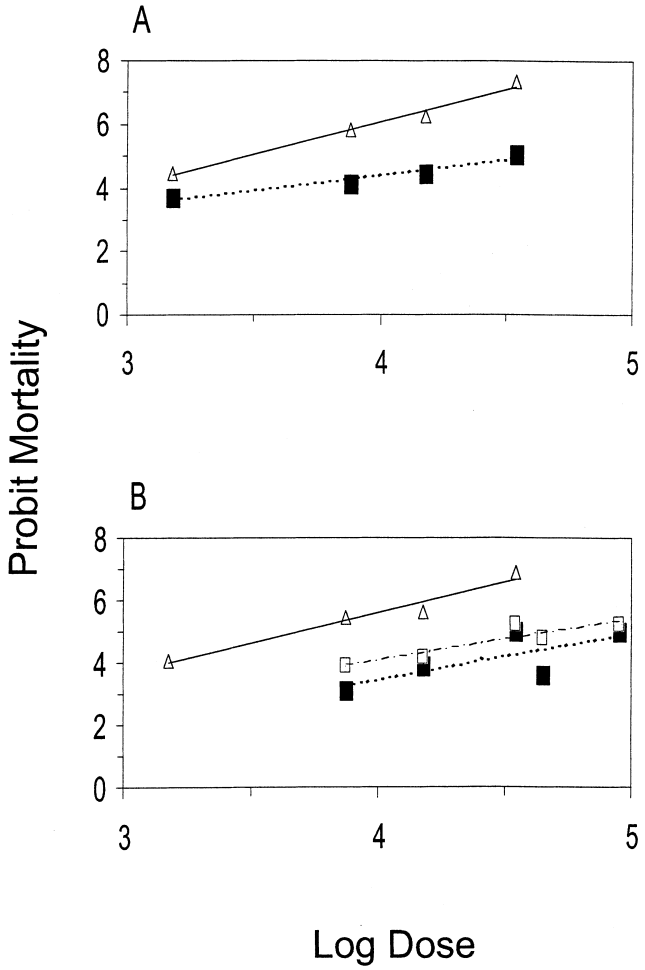

Figure 6. Probit mortality of *T. ni* caterpillars infected with TnSNPV. (a) line C3 (△) and line S3 (■) at generation 17 (data from Milks and Myers, 2000). (b) line C3 (△), subline S3/TnSNPV+ (■) and subline S3/TnSNPV− (□) at generation 27 ($n = 3$ replicates of 10 larvae per dose).

resistance that *T. ni* evolved to TnSNPV (5–22×). This is because the other species in which costs were observed, were selected for a shorter period of time and/or the caterpillars developed lower resistance (*S. frugiperda*: 4.5× resistance following selection with SfNPV for seven generations; Fuxa *et al.*, 1988: *P. interpunctella*: about 2× resistance after 2 years of exposure to PiGV; Boots and Begon, 1993: *A. gemmatalis:* Brazil population: >1000× to AgNPV in 13 generations; US population: 5× to AgNPV in eight generations; Fuxa and Richter, 1998). Furthermore, our inability to detect costs of resistance to TnSNPV was not the result of resistance coadaptation. The difference in the

pupal weight and developmental time of control and selected *T. ni* did not covary with the number of generations of selection (Figs. 1 and 2). There also was no evidence of resistance coadaptation in *Tetranychus urticae* (Helle, 1965), *Culex quinquefasciatus* (Ferrari and Georghiou, 1981), *Musca domestica* (Roush and Plapp, 1982; Whitehead *et al*., 1985) and *Triboleum castaneum* (Beeman and Nanis, 1986) that had evolved resistance to insecticides. However, coadaptation did occur in blowflies resistant to diazinon (*L. cuprina*) (McKenzie *et al*., 1982) and in the soil bacterium *Bacillus subtillis* resistant to the antibiotic rifampicin (Cohan *et al*., 1994).

The tendency for selected cabbage loopers to develop faster may provide some insight into the mechanism of resistance to TnSNPV. Evidence is accumulating that susceptibility of larval lepidoptera to NPV is influenced by the rate at which primary target cells of the virus in the midgut are sloughed off (Washburn *et al*., 1995, 1998; Hoover *et al*., 2000). Individuals that develop quicker may have a faster metabolism and be able to slough off these cells before the virus spreads to other tissues thus increasing their chance of survival.

Stability of resistance to TnSNPV when exposure to the virus stops

The resistance of cabbage loopers to TnSNPV did not decline when exposure to the virus stopped. We believe that resistance was stable because it did not incur any fitness costs and thus there was little selection against resistance allele(s) when exposure to TnSNPV stopped. Resistance to NPVs was costly in *S. frugiperda* and *A. gemmatalis* and both species quickly reverted to susceptibility when exposure to the virus was discontinued (Fuxa and Richter, 1989, 1998).

Why was resistance costly and unstable in *S. frugiperda* and *A. gemmatalis* but not in *T. ni*? Discrepancies among and even within studies that investigate the costs and stability of insect resistance to pathogens are common. Tabashnik *et al*. (1995) derived six isofemale lines from a strain *of P. xylostella* that was highly resistant to *B. thuringiensis*. In five of those lines, resistance declined when exposure to *B. thuringiensis* stopped. However, in one of them, resistance remained high even after >20 generations without exposure to *B. thuringiensis*. Groeters *et al*. (1994) showed that *P. xylostella* resistance to *B. thuringiensis* was costly whereas Tang *et al*. (1997) concluded that it did not cause any reduction in fitness. There also was no cost of resistance to *B. thuringiensis* in *P. interpunctella* (McGaughney and Beeman, 1988). Resistance to pyrethroids incurred fitness costs in *Heliothis virescens* (Campanhola *et al*., 1991) but not in *Helicoverpa armigera* (Daly and Fitt, 1990; Glenn *et al*., 1994). These discrepancies could be the result of different physiological mechanisms and/or mode of inheritance of resistance (Flexner *et al*., 1989; Roush and Daly, 1990).

Resistance to TnSNPV may also have been stable because selection between G1 and 17 removed all susceptible individuals thus preventing any reversion

(e.g., Fuxa and Richter, 1989; Tabashnik *et al*., 1995). At G27, the LD_{50} of sublines S3/TnSNPV+ and S3/TnSNPV− did not differ from one another suggesting that resistance did not evolve between G18 and 26, and that there might have been little genetic variation in susceptibility to TnSNPV left in line S3 when it was subdivided at G17.

Although we did not observe any costs of resistance in terms of pupal weight, developmental time or reproductive success, resistance to TnSNPV could incur other costs that we did not investigate. For example, resistance to TnSNPV could possibly increase the susceptibility of *T. ni* to other pathogens. Fuxa and Richter (1990) showed that *S. frugiperda* that were selected for resistance to an NPV were more susceptible to methyl parathion. Also, resistance to TnSNPV may only be costly when larvae are reared under more stressful conditions such as those in nature. Kraaijeveld and Godfray (1997) observed that *Drosophila melanogaster* that were selected for resistance to an endoparasitoid (*Asobara tabida*) had a poorer survival than control flies when reared in a competitive environment where food was limiting.

If resistance to TnSNPV is not costly then why did Milks and Myers (2000) not observe reciprocal changes in cabbage looper resistance and TnSNPV virulence i.e. coevolution? One possibility is that although the initial TnSNPV stocks used in Experiments 1 and 2 were not clones, their restriction profiles were identical with seven enzymes, and there were no submolar bands suggesting that both samples may have been monomorphic (see Milks and Myers, 2000). Furthermore, little variation for susceptibility in the caterpillar lines may have constrained cabbage loopers to only evolve low to moderate levels of resistance, and this may not have been a sufficiently strong selection pressure on the virus for it to evolve greater virulence. At the end of Experiment 2, the virus was still capable of killing 50% of (22X) resistant caterpillars in spite of not becoming more virulent.

In nature, resistance to the virus and the virulence of TnSNPV are both likely to vary geographically. Furthermore, there are several other factors that could affect the coevolution of cabbage loopers and TnSNPV in nature. For example, *T. ni* are exposed to a variety of pathogens in the wild (Lindgren and Greene, 1984), and a negative correlation between resistance to TnSNPV and any of these pathogens could constrained cabbage looper–TnSNPV coevolution. TnSNPV might also have alternate hosts in nature with some of them being more or less susceptible to the virus. Any or all of these factors could lead to selection mosaics (Thompson, 1994, 1999) and thus the trajectory of cabbage looper–TnSNPV coevolution could vary across landscapes with selection acting on both species, on only one of them or on neither in different communities. Such selection mosaics have been shown in other host–parasite systems including *Drosophila* and its parasitoid (Kraaijeveld and Godfray, 1999), and in a sterilizing trematode and its snail host (Lively, 1999). Finally, this

suggests that it may be necessary to study cabbage loopers and TnSNPV over a wide geographic scale for a prolonged period of time in order to obtain a thorough understanding of their coevolution (see Thompson, 1999).

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