

Genetic analysis of larval host-plant preference in two sibling species of *Helicoverpa*

Qing-Bo Tang^{1,2}, Jin-Wei Jiang³, Yun-Hua Yan¹, Joop J.A. van Loon⁴ & Chen-Zhu Wang^{1*}

¹State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, the Chinese Academy of Sciences, Beijing 100080, China; ²Graduate School of the Chinese Academy of Sciences, Beijing 100039, China; ³College of Plant Protection, Henan Agricultural University, Zhengzhou 450002, China; ⁴Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands

Accepted: 10 November 2005

Key words: *Helicoverpa armigera*, *Helicoverpa assulta*, Lepidoptera, Noctuidae, genetics, feeding preference, *Gossypium hirsutum*, *Capsicum frutescens*

Abstract

The genetic basis of larval host-plant preference was investigated in reciprocal F₁, F₂, and backcrossed generations derived from hybrid crosses between the generalist species *Helicoverpa armigera* (Hübner) and the closely related specialist species *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae). Host-plant preference for cotton [*Gossypium arboreum* L. (Malvaceae)] and pepper [*Capsicum frutescens* L. (Solanaceae)] of fifth-instar caterpillars was tested by using a two-choice leaf-disk assay. *Helicoverpa armigera* and *H. assulta* were significantly different in their feeding preferences, but the difference was not significant in the reciprocal hybrids, which showed there were no maternal/cytoplasmic effects. Comparisons of feeding preference between different groups of females or males demonstrated that the trait was not controlled by sex-linked loci. The distributions of feeding preference index values for crosses that carried similar complements of autosomal genes were not significantly different, whereas crosses with different complements of autosomal genes were associated with significantly different feeding preferences, indicating that feeding preference of the two species for cotton and pepper, respectively, is controlled by autosomal genes. It was found that one major autosomal locus affected this feeding preference, with the *H. armigera*-derived alleles being partially dominant to those carried by *H. assulta*. The genetic analysis of hybrids contributes to understand the evolution of feeding preference in these closely related species.

Introduction

Host-plant selection behavior of larvae of herbivorous insects occurs in three consecutive phases, namely (a) orientation to the food, (b) initial biting response, and (c) continued feeding. Transitions between these phases are essentially take-it-or-leave-it decisions by which the insect either accepts or rejects the plant it has encountered (Schoonhoven et al., 1998). There is broad interest in understanding the evolution of host-plant selection that has given rise to the dominance of specialized feeding habits of phytophagous insects (Futuyma & Peterson, 1985; Via, 1990; Thompson & Pellmyr, 1991; Bernays & Chapman, 1994; Bernays, 2001; Chapman, 2003).

It is particularly interesting to examine the genetic basis of host-plant selection in phytophagous insects, as it is central to theories of evolution, such as sympatric speciation, and has implications for pest management strategies (Futuyma & Peterson, 1985). The genetic basis of oviposition preference has been studied to some extent, and different conclusions have been reached: primarily Z-chromosome linked (Thompson, 1988), non-sex linked (Sheck & Gould, 1995), autosomal (Jaenike, 1987; Hora et al., 2005), monogenic (Carson & Ohta, 1981), or polygenic (Tabashnik et al., 1981; Jaenike, 1987). A few studies have addressed genetic aspects of larval feeding preference in insects (Futuyma et al., 1984; Andersson, 1986; Wallin, 1988), but no detailed genetic data about loci affecting feeding preference were provided.

Pashley (1988) asked whether a reshuffling of the genome may be necessary for a host shift to occur, so that an insect is freed from genetic constraints. Clearly, hybridization brings about a reorganization of the genome involving novel interactions between alleles, between loci, and between

*Correspondence: Chen-Zhu Wang, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, the Chinese Academy of Sciences, Beijing 100080, China. E-mail: czwang@ioz.ac.cn

chromosomal and mitochondrial DNA, and this could conceivably result in host-range expansion or unpredictable changes relative to the parental species (Sheck & Gould, 1995; Hora et al., 2005). Alternatively, hybridization may thwart host-associated speciation, by way of introgression, and may promote stasis of diet breadth (Arnold, 1992; Sheck & Gould, 1995).

The genetic analysis of feeding preference could contribute importantly to studies on the evolution of host-plant selection in phytophagous insects. The sibling species *Helicoverpa armigera* (Hübner) and *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae) are sympatric and serious crop pests in China and other areas of eastern Asia (Chen, 1999). They have a similar appearance, but their host-plant ranges are quite different. One species, *H. armigera*, is a typical generalist; its host-plant range comprises at least 60 plant species, including major crops such as cotton, corn, wheat, soybean, tobacco, and tomato. The host plants of *H. armigera* belong to 47 families of plants, including Malvaceae, Solanaceae, Gramineae, and Leguminosae (Jallow et al., 2004). The closely related *H. assulta* is a specialist with a narrow host-plant range and mainly feeds on plant species in Solanaceae such as tobacco, hot pepper, and several *Physalis* species (Fitt, 1989). Wang & Dong (2001) reported that the reciprocal F₁ generations and backcrossed generations from the two sibling species could be obtained successfully in laboratory conditions, allowing genetic studies of different characters. Wang et al. (2004) reported that there was a clear contrast in feeding preference between the two species, *H. armigera* having a strong feeding preference for cotton and *H. assulta* preferring to feed on pepper rather than on cotton.

In this study, we hybridized the two *Helicoverpa* species and assayed the feeding preferences of the parents, F₁ hybrids, F₂ hybrids, and backcrosses. We compared the strains to determine the inheritance of larval-feeding preference.

Materials and methods

Insects

Larvae of *H. armigera* were collected in 2000 from a cotton field and larvae of *H. assulta* from a tobacco field in Zhengzhou, Henan Province, China. Colonies were maintained as continuous cultures in the laboratory under controlled photoperiod (L16:D8) and temperature (27 ± 1 °C). Adults were supplied with a 10% solution of honey in water. Larvae of both species were reared on an artificial diet (Wu & Gong, 1997). The artificial diet was prepared from the following ingredients: wheat germ (150 g), yeast powder (30 g), methyl-p-hydroxybenzoate (2 g), sorbic acid (1 g), L-ascorbic acid (3 g), linoleic acid (1 ml), agar (14 g), and distilled water (744 ml). Insects were sexed at the pupal stage. The degree of inbreeding of these cultures was reduced by

introducing second-generation specimens collected from the field into the culture once a year. After these introductions, average pupal size increased, indicating that mating between insects from the field and from the lab colony had occurred.

Plants

The plants used in the experiments were cotton [*Gossypium arboreum* L., 'Zhong-12' (Malvaceae)], which is a common host plant of *H. armigera* in the field, and pepper [*Capsicum frutescens* L., 'Nongyan-5' (Solanaceae)], which is a host plant of *H. assulta* in the field. Both plants were field grown at the Crops Research Station of Henan Agricultural University, Zhengzhou, Henan Province, and watered as needed. No chemical sprays were applied to the plants from 2 weeks before and during the experiments.

Inter-specific crosses

Each cross was obtained with 20 pairs of moths kept in a paper cylinder (15 cm diameter and 15 cm height; Xinxing filter paper, Fuyang Co., Hangzhou, China). When female moths began to produce eggs on the filter paper, the filter paper was removed to collect the eggs and replaced by a new one each day. The percentages of females that mated under these conditions ranged from 48% and 67% for *H. assulta* and *H. armigera*, respectively, to 10–12% for the reciprocal hybrid crosses. For the backcrosses, percentages of mating ranged between 45% and 64%. For each cross, 4–5 replicate cages were set up and the eggs obtained were pooled, and hatched larvae were reared on the same artificial diet as described previously.

Because fertile female offspring from the cross *H. armigera* ♀ × *H. assulta* ♂ (abbreviated as RS) was unavailable, five backcrosses and one F₂ cross were obtained. In August 2003, the parentals, F₁, F₂, and backcrossed offspring with various combinations of *H. armigera*-derived and *H. assulta*-derived genomes were produced and tested. This experiment provided data on maternal/cytoplasmic, Z-chromosomal, W-chromosomal, and autosomal effects on feeding behavior based on the comparisons of chromosomal contribution among crosses (see Table 1 for crosses studied and their abbreviations).

Two-choice leaf-disk bioassay

The procedure for testing feeding preference was a modification of that described by Jermy et al. (1968). Two-choice tests were set up in glass Petri dishes of 12 cm diameter for testing fifth-instar larvae that had been starved for 6–8 h. Leaves of the two plant species from which leaf disks (diameter 10 mm) were taken were the third or the fourth leaf from the growing tip of the plant, and at that time, the cotton and the pepper were about 16 weeks and 13 weeks, respectively, since sowing.

Table 1 Comparisons of chromosomal contribution among crosses derived from *Helicoverpa armigera* and *Helicoverpa assulta* and abbreviations used for the different crosses (in each cross, the female parent is given first)

Category	Parents	Offspring designation	% <i>H. armigera</i> -derived genes in female offspring			% <i>H. armigera</i> -derived genes in male offspring	
			Z chromosomes	W chromosome	Autosomes	Z chromosomes	Autosomes
Parental species	<i>H. armigera</i>	RR	100	100	100	100	100
	<i>H. assulta</i>	SS	0	0	0	0	0
F ₁	RR × SS	RS				50	50
	SS × RR	SR	100	0	50	50	50
F ₂	SR × SR	F ₂	50	0	50	75	50
Backcross to RR	RR × RS	BCA	50	100	75	75	75
	RR × SR	BCE	50	100	75	75	75
	SR × RR	BCG	100	0	75	100	75
Backcross to SS	SS × RS	BCB	50	0	25	25	25
	SR × SS	BCF	0	0	25	50	25

For humidity control, a moist sheet of filter paper (12 cm in diameter) was placed at the bottom of each Petri dish. Four leaf disks punched from the leaves of each of the two plant species were arranged in an ABABABAB fashion around the edge of the Petri dish. This arrangement was modified from our earlier study with only two leaf disks of each plant (Wang et al., 2004), and ensured that an active larva had an equal chance of encountering leaf disks of both species.

Insects to be tested were removed from the culture at the end of the fourth instar in the non-feeding premolt stage. They were placed in isolation without food and starved for about 6–8 h after molting into the next instar and then tested. To start the test, a single larva was placed in the center of the dish and leaf consumption was observed at approximately hourly intervals thereafter. Petri dishes were placed under evenly distributed fluorescent lights at a temperature of 27 ± 1 °C. The assay was ended when ca. 50% of either of the two disk types (A or B) had been consumed, and subsequently, the disk area of each plant species consumed was measured using transparency film (PP2910, 3M Co.) with a 1-mm² grid and recorded. Each larva was tested only once and was transferred after the feeding bioassay to a 25-ml glass tube (one larva per tube) and reared on the artificial diet to pupation, after which its sex was determined.

Data analysis

The percentage of total consumption of each plant (choice index) was calculated for each larva as a measure of its feeding preference, as follows:

Percent total consumption of cotton (Pc) = (consumption of cotton)/(consumption of cotton + pepper)

and

Percent total of pepper (Pp) = (consumption of pepper)/(consumption of cotton + pepper)

Mean, standard deviation, and standard error of Pc or Pp were calculated for females and males of the parent species and each cross. Because the data were not normally distributed, non-parametric statistics were applied. All data are presented here as percentage consumption of cotton or pepper leaf disks. A two-tailed Wilcoxon matched pairs signed ranks test was used to test the significance of difference in consumption between cotton and pepper leaf disks within each parent species and cross. To compare different crosses, a Kruskal–Wallis test with multiple comparisons was applied to the raw data. Differences in feeding preferences among crosses were considered to be statistically significant when $P < 0.05$ (two-tailed). To estimate the relationship between feeding preference and the percentage of *H. armigera*-derived autosomal genes, we regressed the mean of Pc on percentage of *H. armigera*-derived autosomal genes at the population level (each cross was considered as one population). We could not regress at the individual level because feeding preference in the crosses derived from backcrossing to *H. assulta* was segregating significantly. All data were analyzed using the statistical package SPSS version 10.0 for Windows (SPSS Inc., Chicago, IL, USA) (Lu, 2000).

Results

Feeding preference of parents and crosses

The host-plant feeding preference of females and males in each cross are shown in Figures 1 and 2, respectively. The differences in feeding preferences for cotton and pepper were significant in *H. armigera*, *H. assulta*, RS, and backcrosses obtained from both reciprocal F₁ crosses to *H. armigera* (Figures 1 and 2). The mean percentages of cotton leaf area consumed in *H. armigera* females and males were 89.83 ± 0.02 and 93.02 ± 0.01 , respectively. The reverse preference was found for *H. assulta* females and males that

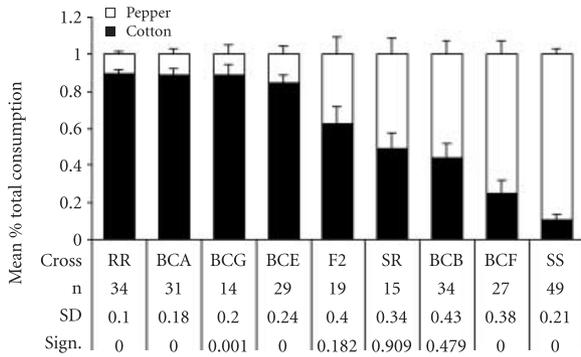


Figure 1 Feeding preference for leaf disks of cotton (black bar) and pepper (white bar) by female fifth-instar caterpillars in each cross derived from *Helicoverpa armigera* and *Helicoverpa assulta*. Graphs depict the consumption of two host plants during the choice test (each expressed as percent of total consumption) in each cross. Columns represent mean + SE of the choice index for the larvae tested. Cross = each cross generated from *H. armigera* and *H. assulta*, see Table 1 for abbreviations. n = number of larvae tested. SD = Standard deviation of the mean in each cross. Sign. = probability that differences in consumption of either leaf disk type in the two-choice test is due to chance (two-tailed Wilcoxon matched pairs signed ranks test).

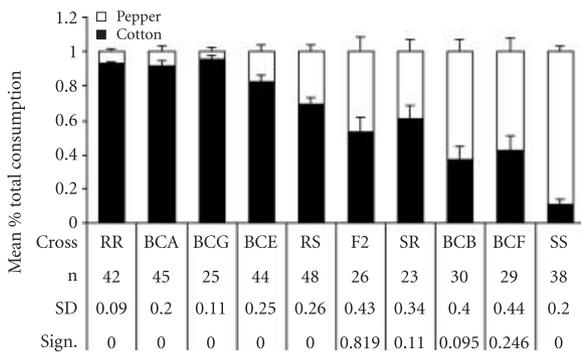


Figure 2 Feeding preference between leaf disks of cotton (black bar) and pepper (white bar) by male fifth-instar caterpillars in each cross derived from *Helicoverpa armigera* and *Helicoverpa assulta*. Legend as for Figure 1. See Table 1 for abbreviations of crosses.

consumed only $10.47 \pm 0.03\%$ and $10.71 \pm 0.03\%$, respectively. The mean percentages of cotton or pepper leaf disks consumed in either females or males of F₁, F₂, and backcrossed generations were intermediate to those of the parent species, with the exception of BCG males (Figures 1 and 2).

Maternal effects

The caterpillars of *H. armigera* and *H. assulta*, which differed significantly in feeding preference for cotton ($\chi^2 = 137.267$, d.f. = 1, $P < 0.001$), were crossed reciprocally to determine

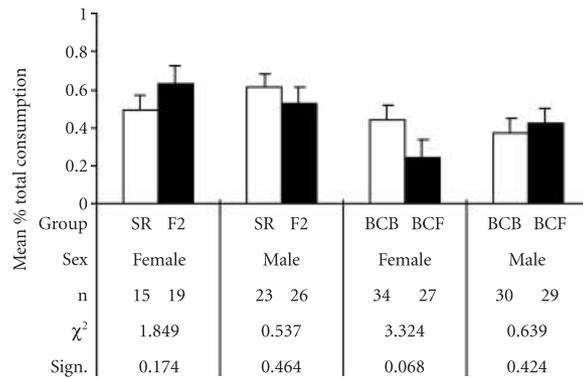


Figure 3 Effect of Z chromosome on feeding preference for cotton leaf disks in different pairs of crosses between *Helicoverpa armigera* and *Helicoverpa assulta*. Note that every two groups of larvae compared only differed by at least 25% in the origins of loci on the Z chromosome. Group = each pair of groups among which feeding preferences on cotton leaf disks were compared. Sex = the sex of larvae in each pair of crosses. n = number of larvae tested. χ^2 = value of Kruskal–Wallis statistic in each pair of crosses. Sign = probability that there is a difference in feeding behavior within each pair of groups.

if there were cytoplasmic or maternal effects on feeding behavior. Because fertile female offspring from the RS was unavailable, only males of F₁ crosses were compared. The difference in feeding preference between the male larvae derived from the RS and the male larvae produced by the reciprocal cross (SR) was not significant ($\chi^2 = 0.537$, d.f. = 1, $P = 0.464$). Thus, maternal/cytoplasmic effects cannot account for the large difference in larval-feeding preference between the two species.

Sex-chromosomal effects

A number of crosses were produced that carried the same cytoplasm and similar complements of autosomal genes, but that differed by at least 25% in the origins of loci on the Z chromosome. There are four such pairs of groups, viz., SR females and F₂ females, SR males and F₂ males, BCB females and BCF females, and BCB males and BCF males; in none of them do the two groups differ significantly in feeding preference (Figure 3), which indicates that there is no distinct Z-chromosomal effect.

The results also demonstrate that the difference in feeding preference between the two species was not determined by loci on the W chromosome. As seen from Figure 4, each of the three pairs of groups, *H. armigera* females and *H. armigera* males, *H. assulta* females and *H. assulta* males, and BCG females and BCG males, differed by the fact that only the females had a W chromosome and were similar in their sets of autosomes and Z chromosome, but no significant differences in feeding preference between females

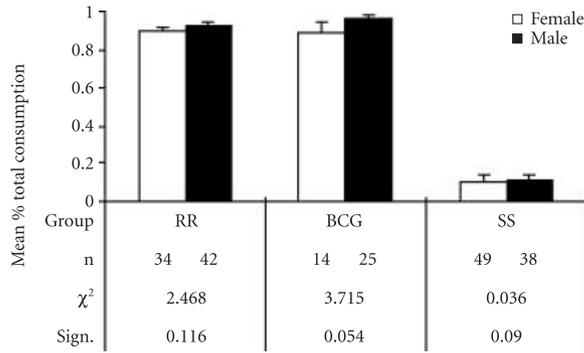


Figure 4 Effect of W chromosome on feeding preferences for cotton within different pairs of groups. Legend as for Figure 3. White bars represent values for females, black bars those for males.

and males in each group were observed. Therefore, this experiment provides no evidence that either the Z chromosome or the W chromosome make a statistically detectable contribution to variation in feeding behavior.

Autosomal effects

According to the percentage of *H. armigera*-derived autosomal genes (Table 1), the two parent species and the eight crosses can be classified into five groups: *H. armigera* with 100% of *H. armigera* autosomal loci; BCA, BCE, and BCG with 25% difference from *H. armigera* in the origin of autosomal genes; RS, SR, and F_2 with 50% difference; BCB and BCF with 75% difference; and *H. assulta* with 100% difference. The distributions of crosses belonging to different groups demonstrated significantly different feeding preferences, for example, females of all crosses ($\chi^2 = 116.429$, d.f. = 9, $P < 0.001$), males of all crosses ($\chi^2 = 151.611$, d.f. = 9, $P < 0.001$), females of SR, F_2 , BCB, and BCF ($\chi^2 = 10.997$, d.f. = 3, $P = 0.012$), males of RS, SR, F_2 , BCB, and BCF ($\chi^2 = 13.798$, d.f. = 4, $P = 0.008$). The distributions of feeding preference of crosses belonging to the same group were not significantly different, for example, females of BCA, BCE, and BCG ($\chi^2 = 0.539$, d.f. = 2, $P = 0.764$), females of SR and F_2 ($\chi^2 = 1.849$, d.f. = 1, $P = 0.174$), females of BCB and BCF ($\chi^2 = 3.324$, d.f. = 1, $P = 0.068$), males of BCG and BCA ($\chi^2 = 3.139$, d.f. = 1, $P = 0.076$), males of RS, SR, and F_2 ($\chi^2 = 1.402$, d.f. = 2, $P = 0.496$), and males of BCB and BCF ($\chi^2 = 0.639$, d.f. = 1, $P = 0.424$). With the percentages of *H. armigera*-derived autosomal genes decreasing from *H. armigera* to F_1 generations, backcrossed generations, and to *H. assulta*, the means of the choice indices P_c of these generations decreased accordingly (Figures 1 and 2). In addition, the mean of the choice index (P_c) of each cross in females and males was significantly positively correlated with the percentage of

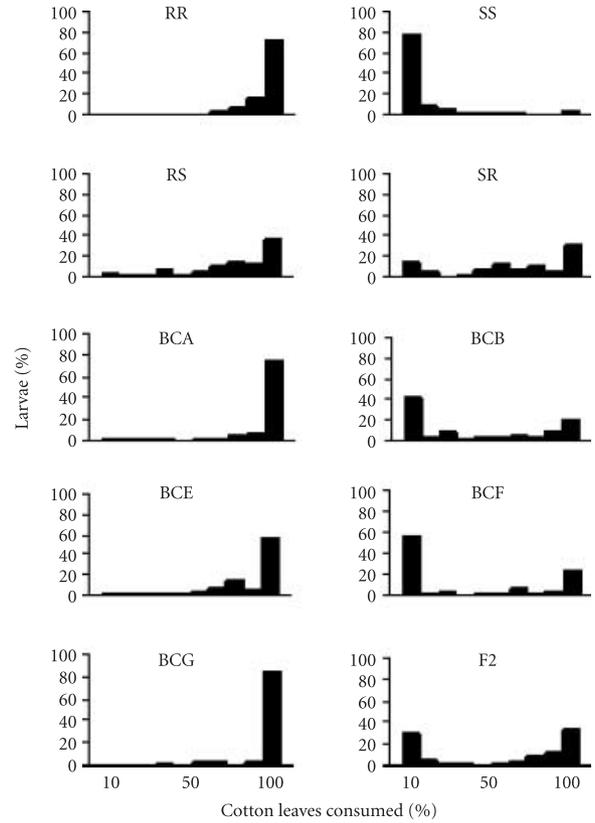


Figure 5 The distributions of proportion of cotton leaf disks consumed by fifth-instar caterpillars of *Helicoverpa armigera*, *Helicoverpa assulta*, F_1 , F_2 , and backcrosses.

H. armigera-derived autosomal genes at the population level ($r = 0.954$, $P < 0.001$ in females and $r = 0.959$, $P < 0.001$ in males). Thus, our results demonstrate that feeding preference of *H. armigera* and *H. assulta* for cotton and pepper, respectively, is determined by an autosomal locus or possibly several loci.

Discussion

We found that the *H. armigera*-derived alleles were partially dominant to those carried by *H. assulta*. Distributions of feeding preference for cotton of both reciprocal F_1 crosses resembled *H. armigera* more than those of *H. assulta* (Figure 5), although the distributions of feeding preferences for both RS and SR hybrids differed significantly with those for *H. armigera* ($\chi^2 = 29.93$, d.f. = 1, $P < 0.001$ and $\chi^2 = 28.904$, d.f. = 1, $P < 0.001$, respectively). Male RS and SR hybrids displayed a similar distribution of feeding preferences ($\chi^2 = 0.545$, d.f. = 1, $P = 0.461$). Moreover, feeding preference distributions of backcrosses obtained from both reciprocal F_1 crosses to *H. armigera* are more

similar to *H. armigera* than to *H. assulta* (Figure 5). BCA, BCE, and BCG were different from *H. armigera* by 25% in *H. armigera*-derived autosomal genes, but *H. armigera*, BCE, and BCA had similar feeding preferences ($\chi^2 = 4.986$, d.f. = 2, $P = 0.083$) and the choice index of BCG ($P_c = 0.94$) was even higher than that of *H. armigera* ($P_c = 0.92$).

The question may be asked how many autosomal loci are involved in controlling the difference in feeding preference between the two *Helicoverpa* species. There were two distinct classes in the distribution of feeding preferences in each of the three crosses F_2 , BCB, and BCF, whereas only one class was observed in the other three backcrosses, BCA, BCE, and BCG (Figure 5). BCB and BCF were the backcrosses that were obtained by crossing the F_1 reciprocally to *H. assulta*, whereas BCA, BCE, and BCG were obtained by crossing the F_1 reciprocally to *H. armigera*. Meanwhile, the variation around the mean choice indices for F_2 , BCB, and BCF is higher than for the parent species, the reciprocal F_1 generations, BCA, BCE, and BCG (Figures 1 and 2). These results are expected if at least one major locus affecting feeding preference would segregate.

Other evidence for the involvement of at least one major locus in the difference in host-plant preference between *H. armigera* and *H. assulta* is presented in Figure 5. Of the BCB larvae 45.1%, of BCF larvae 58.2%, and of F_2 larvae, 35.7% displayed 0–20% of leaf-disk consumption from cotton. These proportions are close to the 53.55%, 53.55%, and 31.9% of larvae that would be expected for the three respective crosses if one major autosomal locus affecting feeding preference would segregate. Expected values are based on the observation that for 0% of the larvae in *H. armigera*, 86.6% of the larvae in *H. assulta*, and 20.5% of the larvae in SR, 0–20% of leaf disk consumption was from cotton.

Larval-feeding preference might have important fitness consequences. Food plants available to neonate larvae are often thought to be limited to those present close to the oviposition sites selected by the female parent (Singer, 1971; Andersson, 1986). However, interplant movement in later instars of *Helicoverpa* was found to be extensive, implying that active food selection can be exerted in the larval stage (Cunningham et al., 2001). Hanson (1976) studied two species of nymphalid butterflies and found that their hybrid was intermediate in feeding preference for cherry. Futuyma et al. (1984) showed that incipient divergence of *Alsephila pometaria* in host utilization occurs via differences in larval preference but not larval performance on difference hosts. Different strains of *Drosophila melanogaster* larvae showed genetically determined differences in their feeding-preference behavior (Andersson, 1986; Wallin, 1988). Feeding preference of these strains of *D. melanogaster*

was not sex linked, neither were there maternal effects (Andersson, 1986). The behavior of F_2 flies from reciprocal crosses between two strains of *Drosophila tripunctata* demonstrated that genetic variation for feeding preference was autosomal and largely additive (Jaenike, 1985). Pappas et al. (2002) concluded that genetically determined polymorphisms in morphology and feeding preference exist in *Galerucella nymphaeae*, resulting in differential performance. Also, our finding that larval feeding preference is largely determined by autosomal genes agrees with studies on larval performance of *Papilio* by Thompson et al. (1990) and of *Heliothis* by Sheck & Gould (1993). Whether larval feeding preference and larval performance are controlled by the same loci requires additional experimental work.

Two primary reasons may explain why there is relatively little information on the genetics of larval feeding preference of phytophagous insects. First, Singer (1971) argued that neonate larvae in the field lack sufficient power of movement to leave the plant on which the eggs were laid and can exercise no food-plant preference. Second, variation in feeding preference in phytophagous insects could result from ecological interactions, developmental or physiological state, and various types of experience (Bernays & Chapman, 1994), making it difficult to determine to what extent genetic factors contribute to the variation of feeding preference observed. In the present study, prior to being tested, the larvae of the two insect species had been raised on artificial diet only, eliminating the involvement of experience with a particular host plant. The feeding preferences we determined were therefore innate. As described by Lu et al. (2001), the environmentally induced variation in larvae should be the same for all offspring grown at the same time on the same hosts, regardless of their parentage; thus, any additional variation among larvae from a specific cross can be attributed to genetic differences. To assess whether the larval feeding preference on host plants were stable in different plant-development stages, the feeding preferences of the two *Helicoverpa* species to cotton and pepper were tested on three consecutive occasions in July, August, and September. The results showed that both *H. armigera* and *H. assulta* had consistent preferences over the 3 months (Table 2), suggesting that the possible effects of differences between plants grown in these 3 months on feeding preference were not important. Although leaves of cotton and pepper plants are not preferred foods of *H. armigera* and *H. assulta*, which both prefer the fruiting organs of these plants, the purpose of this study was the genetic analysis of preference behavior, for which we took advantage of the significant contrast in feeding preference between cotton and pepper leaf material. Larger sample sizes would be needed to provide a better estimate of the variances in the backcross and F_2 generations for comparison with the

Table 2 Comparison of feeding preference for cotton in *Helicoverpa armigera* and *Helicoverpa assulta* among different months. Pc is the choice index for cotton in each cross. Sign. denotes the probability that feeding preference for cotton in *H. armigera* or *H. assulta* differed among the three months (Kruskal–Wallis test)

Cross	<i>H. armigera</i>			<i>H. assulta</i>		
	July	Aug.	Sept.	July	Aug.	Sept.
n	19	98	18	22	97	18
Pc	0.92	0.93	0.97	0.08	0.1	0.13
SD	0.1	0.09	0.07	0.13	0.19	0.26
χ^2	0.54			0.72		
Sign.	0.77			0.70		

F₁ generation (Thompson, 1988). As in many inter-specific crosses, however, it was difficult to obtain viable and fertile F₁, F₂, and backcrossed generations. We managed to obtain only a limited number of SR individuals from *H. assulta* ♀ × *H. armigera* ♂ and could not obtain female individuals from RS.

Gustation has a key role in food acceptance or rejection by phytophagous insects (Chapman, 2003). Food-plant recognition in lepidopterous larvae is predominantly governed by the activity of eight taste neurones present in two sensilla styloconica located on each maxilla, and even closely related species have different taste systems (Schoonhoven & van Loon, 2002). We found differences in electrophysiological responses of the galeal styloconic sensilla of final instar caterpillars of these two *Helicoverpa* species to stimulation by cotton and pepper leaf saps (QB Tang et al. unpubl.).

In summary, the hybridization approach we took revealed that feeding behavior of hybrid larvae and backcrosses differed from that of the two parent species and demonstrated significant heritability. The results are congruent with the explanation that the differences in larval-feeding preference between the two species is largely determined by at least one major autosomal locus with the *H. armigera*-derived alleles being partially dominant to those carried by *H. assulta*.

As our genetic analysis suggests at least one major gene was involved in determining the feeding preference of *H. armigera* and *H. assulta*, this opens up the possibility of molecular analysis. One of the promising approaches is the development of a genomic map and a search for linkage groups that are associated with 'feeding preference' phenotypes. The molecular genetic aspects of such an approach are becoming less formidable as genomic technology becomes more efficient and allows the identification of quantitative trait loci (Lu et al., 2001).

Acknowledgements

We thank Steph Menken and Hans Breeuwer for their useful comments on this manuscript in its early stages, Ji-Sheng Ma and Guo-Hui Yuan for their help in plant cultivation and laboratory facilities, Yue-Li Jiang, Yan-Zheng Hu, and Jing-Fang Zhang for assistance in the experiments, Li-Hua Ma for providing cotton seeds, and Li Feng for assistance in insect rearing. This work was supported by the National Natural Science Foundation of China (grant no. 30330100 and 30471148) and the State Key Laboratory of Integrated Management of Pest Insects and Rodents (grant no. Chinese IPM0503).

References

- Andersson A (1986) Genetic influence on foraging behaviour in *Drosophila melanogaster* larvae. *Hereditas* 105: 229–231.
- Arnold ML (1992) Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics* 23: 237–261.
- Bernays EA (2001) Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annual Review of Entomology* 46: 703–727.
- Bernays EA & Chapman RF (1994) *Host-Plant Selection by Phytophagous Insects*. Chapman & Hall, New York, NY, USA.
- Carson HL & Ohta AT (1981) Origin of the genetic basis of colonizing ability. *Evolution Today. Proceedings of the Second International Congress of Systematic and Evolutionary Biology* (ed. by GGE Scudder & JL Reveal), pp. 365–370. Hunt Institute for Botanical Documentation, Carnegie Mellon University, Pittsburgh, PA, USA.
- Chapman RF (2003) Contact chemoreception in feeding by phytophagous insects. *Annual Review of Entomology* 48: 455–484.
- Chen YX (1999) *Fauna Sinica*, Vol. 16. Insecta, Lepidoptera, Noctuidae. Science Press, Beijing, China (in Chinese).
- Cunningham JP, West SA & Zalucki MP (2001) Host selection in phytophagous insects: a new explanation for learning in adults. *Oikos* 95: 537–543.
- Fitt GP (1989) The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* 34: 17–52.
- Futuyma DJ, Cort RP & van Noordwijk I (1984) Adaptation to host plants in the fall cankerworm (*Alsophila pometaria*) and its bearing on the evolution of host affiliation in phytophagous insects. *The American Naturalist* 123: 287–296.
- Futuyma DJ & Peterson SC (1985) Genetic variation in the use of resources by insects. *Annual Review of Entomology* 30: 217–238.
- Hanson FE (1976) Comparative studies on induction of food choice preference in lepidopterous larvae. *Symposia Biologica Hungarica* 16: 71–77.
- Hora KH, Roessingh P & Menken SBJ (2005) Inheritance and plasticity of adult host acceptance in *Yponomeuta* species: implications for host shifts in specialist herbivores. *Entomologia Experimentalis et Applicata* 115: 271–281.
- Jaenike J (1985) Genetic and environmental determinants of food preference in *Drosophila tripunctata*. *Evolution* 39: 362–369.

- Jaenike J (1987) Genetics of oviposition-site preference in *Drosophila tripunctata*. *Heredity* 59: 363–369.
- Jallow MFA, Cunningham JP & Zalucki MP (2004) Intra-specific variation for host plant use in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae): implications for management. *Crop Protection* 23: 955–964.
- Jermey T, Hanson FE & Dethier VG (1968) Induction of specific food preference in lepidopterous larvae. *Entomologia Experimentalis et Applicata* 11: 211–230.
- Lu WD (2000) SPSS for Windows: Data Analysis. Publishing House of Electronics Industry, Beijing, China (in Chinese).
- Lu W, Kennedy GG & Gould F (2001) Genetic analysis of larval survival and larval growth of two populations of *Leptinotarsa decemlineata* on tomato. *Entomologia Experimentalis et Applicata* 99: 143–155.
- Pappers SM, van der Velde G, Ouborg NJ & van Groenendael JM (2002) Genetically based polymorphisms in morphology and life history associated with putative host races of the water lily leaf beetle, *Galerucella nymphaeae*. *Evolution* 56: 1610–1621.
- Pashley DP (1988) Quantitative genetics, development and physiological adaptation in host strains of fall armyworm. *Evolution* 42: 93–102.
- Schoonhoven LM, Jermey T & van Loon JJA (1998) *Insect-Plant Biology: From Physiology to Evolution*. Chapman & Hall, London, UK.
- Schoonhoven LM & van Loon JJA (2002) An inventory of taste in caterpillars: each species its own key. *Acta Zoologica Academiae Scientiarum Hungarica* 48: 215–263.
- Sheck AL & Gould F (1993) The genetic basis of host range in *Heliothis virescens*: larval survival and growth. *Entomologia Experimentalis et Applicata* 69: 157–172.
- Sheck AL & Gould F (1995) Genetic analysis of differences in oviposition preferences of *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae). *Environmental Entomology* 24: 341–347.
- Singer MC (1971) Evolution of food-plant preference in the butterfly *Euphydryas editha*. *Evolution* 25: 383–389.
- Tabashnik BE, Wheelock H, Rainbolt JD & Watt WB (1981) Individual variation in oviposition preference in the butterfly, *Colias eurytheme*. *Oecologia* 50: 225–230.
- Thompson JN (1988) Evolutionary genetics of oviposition preference in swallowtail butterflies. *Evolution* 42: 1223–1234.
- Thompson JN & Pellmyr O (1991) Evolution of oviposition behavior and host preference in Lepidoptera. *Annual Review of Entomology* 36: 65–89.
- Thompson JN, Wehling W & Podolsky R (1990) Evolutionary genetics of host use in swallowtail butterflies. *Nature* 344: 148–150.
- Via S (1990) Ecological genetics and host adaptation in herbivorous insects: the experimental study of evolution in natural and agricultural systems. *Annual Review of Entomology* 35: 421–446.
- Wallin A (1988) The genetics of foraging behaviour: artificial selection for food choice in larvae of the fruitfly, *Drosophila melanogaster*. *Animal Behaviour* 36: 106–114.
- Wang CZ & Dong JF (2001) Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera: Noctuidae). *Chinese Science Bulletin* 46: 489–491.
- Wang CZ, Dong JF, Tang DL, Zhang JH, Li W & Qin JD (2004) Host selection of *Helicoverpa armigera* and *H. assulta* and its inheritance. *Progress in Natural Science* 14: 880–884.
- Wu KJ & Gong PY (1997) A new and practical artificial diet for the cotton bollworm. *Entomologia Sinica* 4: 277–282.