

Hybridization between *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera: Noctuidae): development and morphological characterization of F₁ hybrids

X.-C. Zhao^{1,2}, J.-F. Dong¹, Q.-B. Tang^{1,2}, Y.-H. Yan¹,
I. Gelbic³, J.J.A. Van Loon⁴ and C.-Z. Wang^{1*}

¹State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China: ²Graduate School of the Chinese Academy of Sciences, Beijing 100039, China: ³Department of Morphology, Institute of Entomology AS CR, 370 05 Ceske Budejovice, Czech Republic: ⁴Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands

Abstract

Reciprocal hybridizations between *Helicoverpa armigera* (Hübner) and *Helicoverpa assulta* (Guenée) were studied. The cross between females of *H. armigera* and males of *H. assulta* yielded only fertile males and sterile individuals lacking an aedeagus, valva or ostium bursae. A total of 492 larvae of the F₁ generation were obtained and 374 of these completed larval development and pupated. Only 203 pupae were morphologically normal males, the remaining 171 pupae were malformed. Larvae and pupae that gave rise to morphologically abnormal adults exhibited longer development times. Sterility was not only associated with malformed external sex organs, but also a range of abnormalities of the internal reproductive system: (i) loss of internal reproductive organs, (ii) with one to three copies of an undeveloped bursa copulatrix; or (iii) with one or two undeveloped testes. Normal male hybrid adults showed higher flight activity in comparison with males of both species. In contrast, the cross between females of *H. assulta* and males of *H. armigera* yielded morphologically normal offspring (80 males and 83 females). The interaction of the Z-chromosome from *H. assulta* with autosomes from *H. armigera* might result in morphological abnormalities found in hybrids and backcrosses, and maternal-zygotic incompatibilities might contribute to sex bias attributed to hybrid inviability.

Keywords: *Helicoverpa armigera*, *Helicoverpa assulta*, Noctuidae, Lepidoptera, interspecific hybrid, morphology, sterility

*Author for correspondence

Fax: +86 10 6256 5689

E-mail: czwang@ioz.ac.cn

Introduction

Hybridization studies between closely related species mainly serve to investigate the genetic bases of morphological, behavioural and physiological traits and the genetic incompatibilities resulting in hybrid sterility and inviability (Gruła & Taylor, 1980; Coyne & Orr, 1989; Turelli & Orr, 2000; Presgraves, 2002). Such studies contribute not only to our knowledge of genetic architecture and speciation, but might also yield spin-offs useful in pest control (Downes, 1959; Knippling, 1960; Laster, 1972; Coyne & Orr, 1989; Presgraves, 2002). Hybrids carrying two inherited lethal or sterile factors as a result of crossing two related species or races could be used to reduce populations of parent species (Knippling, 1960).

Many heliothine moths, *Heliothis* and *Helicoverpa* species (Lepidoptera: Noctuidae), are important pests in agriculture worldwide (Fitt, 1989). Interspecific hybridizations of heliothine moths in the laboratory have been documented (Hardwick, 1965; Laster, 1972; Laster *et al.*, 1988; Degrugillier & Newman, 1993; Laster & Sheng, 1995; Laster & Hardee, 1995; Wang & Dong, 2001). For example, hybridization between females of *Heliothis subflexa* (Guenée) and males of *Heliothis virescens* (Fabricius) produced fertile F₁ females and sterile males. Backcrosses of F₁ females with male *H. virescens* produced fertile females and sterile males (Laster, 1972). The sterility caused by interspecific hybridization has been successfully used as a genetic means of control in the field against *H. virescens* (Proshold, 1983). The possible mechanisms of this hybrid sterility are a deficiency of mitochondria derived from the female of *H. subflexa* (Miller *et al.*, 1986), or incompatibility caused by interactions between microorganisms and genetic material in the nucleus of the paternal generation (Krueger *et al.*, 1993). Hybridization of *Helicoverpa punctigera* (Wallengren) with *Helicoverpa zea* (Boddie) and *Helicoverpa armigera* (Hübner) produced no offspring (Hardwick, 1965). However, fertile offspring were produced by *H. zea* mated with *H. armigera* and *Helicoverpa assulta* (Guenée) (Hardwick, 1965; Degrugillier & Newman, 1993). An attempt to find sterile hybrids for *H. zea* control failed (Laster & Sheng, 1995).

The cotton bollworm, *H. armigera*, and the oriental tobacco budworm, *Helicoverpa assulta* (Guenée), are closely related species and are sympatrically distributed in China (Chen, 1999). It is not feasible to distinguish these species in the field based solely on morphological features of eggs, larvae, and pupae. Their phenology overlaps from middle May to middle October. However, *H. armigera* is a polyphagous species feeding on more than 60 crop species such as cotton, corn and soybean, while *H. assulta* is an oligophagous species using only some solanaceous species such as tobacco and hot pepper (Fitt, 1989; Chen, 1999). Interspecific hybrids between *H. armigera* and *H. assulta* have been obtained (Wang & Dong, 2001). The female copulation rate in crossing female *H. assulta* with male *H. armigera* was 8.8% and that of female *H. armigera* × male *H. assulta* was 7.1%. The F₁ hybrids of female *H. assulta* and males *H. armigera* are fertile with a 1:1 sex ratio. The F₁ hybrids derived from female *H. armigera* × male *H. assulta* yielded fertile males but no females (Wang & Dong, 2001). The latter F₁ hybrid males showed heterosis in pupal weight and adult activity, and the backcross line of these F₁ hybrids with female *H. armigera* had both female and male offspring but with a skewed sex ratio and male bias (1:4), which

provides a potential method of genetically controlling *H. armigera*.

The aim of the present study was to determine the development, morphology, and flight activity, under laboratory conditions, of F₁ hybrids between *H. armigera* and *H. assulta*. Abnormal individuals were identified in the F₁ hybrids of female *H. armigera* × male *H. assulta* and the characteristics of the reproductive system of these abnormal individuals are described. The causes of the production of abnormal individuals and failure to produce hybrid females in one of the reciprocal crosses and the potential of the findings for pest control are discussed.

Materials and methods

Insect rearing

Helicoverpa armigera and *H. assulta* used in the hybridization experiments were taken from established colonies of both species that originated from larvae collected from cotton and tobacco fields, respectively, in Zhengzhou, Henan province of China. Both species were separately reared for more than ten generations in climate-controlled chambers at 27 ± 1°C, 16L:8D photoperiod and 70 ± 10% relative humidity. Adults were supplied with 10% honey solution in water as food. Larvae of both species were reared on an artificial diet with wheat germ as the main ingredient (Wu & Gong, 1997) in a 25 ml glass tube (one larva per tube). Adults from the basic rearing colony were used for all experiments. Individuals were separated into males and females at the pupal stage.

Interspecific hybridization

The hybridization scheme for *H. armigera* and *H. assulta* is shown in fig. 1. Reciprocal crosses of female *H. armigera* × male *H. assulta* and female *H. assulta* × male *H. armigera* were undertaken to produce two lines of F₁ hybrids. The various backcrosses were designed to test the fertility of F₁ hybrids.

Each cross was made with 20 pairs kept in a cylindrical paper box (15 cm diameter and 20 cm height). Eggs were

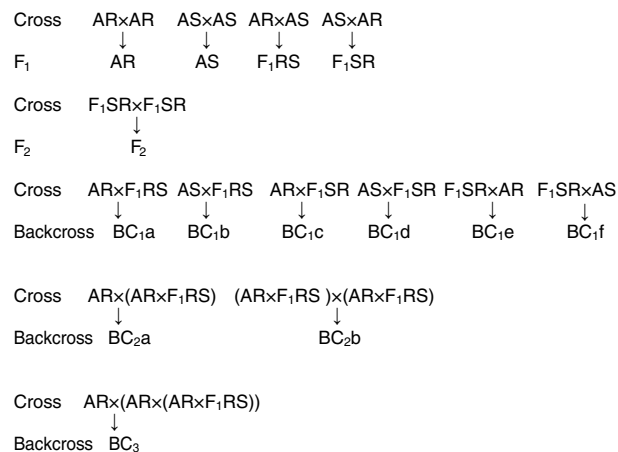


Fig. 1. Female and male crossing scheme employed to produce hybrids between *Helicoverpa armigera* (AR) and *H. assulta* (AS) and backcrosses.

collected daily and hatched larvae of F₁ generations were reared under the conditions described above.

For the experimental F₁ generations, the numbers of larvae and pupae and their developmental time were recorded. Morphological features of the external and internal reproductive organs of adult moths were examined using a Wild M3 (Wild, Heerbrugg, Switzerland) stereomicroscope at 10× magnification. The genitalia of pupae were also examined.

Flight ability test

The flight ability of males of *H. armigera*, *H. assulta*, and of F₁ hybrids of female *H. armigera* × male *H. assulta* was measured using the bioassay described by Cheng *et al.* (2002). Virgin 3-day-old males were tethered on the rod of a flight-mill by using Super glue 502 (Guangdong Aibida Adhesives Co. Ltd, Guangzhou, China) for 24 h in darkness at 22 ± 1°C. Flight time, the number of rotations, distance and speed were automatically recorded by a computer.

Statistical analyses

All statistical analyses were performed using the SPSS 11.01 (2001) software package. Diameter of testes, developmental times and parameters of insect flight ability were subjected to analysis of variance (one-way ANOVA) and the least significant difference (LSD) was used to assess differences at $P = 0.05$ among *H. armigera*, *H. assulta* and their F₁ hybrids. The *t*-test was used to determine significance of differences in developmental times between males, females or abnormal individuals of *H. armigera*, *H. assulta* and their F₁ hybrids. The Chi-square test was used to compare ratios of female, male, and abnormal individuals with expected values.

Results

The F₁ hybrid of female *H. armigera* × male *H. assulta*

Adults of the F₁ hybrid of *H. armigera* females × *H. assulta* males were divided into normal and abnormal individuals

according to their external reproductive organs. The normal hybrids were all fertile males, with a typical reproductive system similar to that of their male parent, and were able to cross with both *H. armigera* and *H. assulta* females to produce backcross lines (fig. 2a). The abnormal hybrids were sterile lacking an aedeagus, valva or ostium bursae on associated genital segments, but with a tuba analis and an uncus (fig. 2b). No female F₁ hybrids were produced. The ratio of normal males to abnormal individuals was 1:0.84, which was not significantly different from 1:1 ($\chi^2 = 2.78$; $P = 0.098$). Table 1 shows that three types of internal reproductive system were observed in abnormal F₁ hybrids: (i) individuals lacking internal reproductive organs; (ii) individuals with one to three copies of an undeveloped bursa copulatrix, lacking accessory glands (fig. 3d), and (iii) individuals with one or two undeveloped testes and lacking seminal vesicles, ductus ejaculatorius and accessory glands (fig. 3c). The diameter of the undeveloped testes was $922 \pm 53.2 \mu\text{m}$ (means ± SEM, $n = 11$), which was significantly smaller ($F_{3,36} = 30.0$; $P < 0.05$) than that of the normal F₁ males ($1836 \pm 119 \mu\text{m}$), *H. armigera* males ($1888 \pm 87.2 \mu\text{m}$) and *H. assulta* males ($1636 \pm 82.1 \mu\text{m}$).

Abnormal F₁ hybrids were also identified in the pupal stage. They were divided morphologically into two distinct types. Type A were abnormal pupae that lacked a reproductive organ opening and with the eighth abdominal segment exhibiting morphological features intermediate between male and female (fig. 4b). Type B were abnormal pupae that also lacked a reproductive organ opening but with two projections on the eighth abdominal segment (fig. 4c). Figure 4a and d show the reproductive organ opening and the eighth abdominal segment of typical male and female pupae. In the adults that eclosed from type A abnormal pupae, all three abnormal varieties of internal reproductive system were observed, but in the adults that eclosed from type B abnormal pupae only the type with one to three copies of an undeveloped bursa copulatrix was found.

More than 80 females of *H. armigera* and more than 80 males of *H. assulta* were used for hybridization. A total of 492 larvae of the F₁ hybrid generation were obtained (table 2),

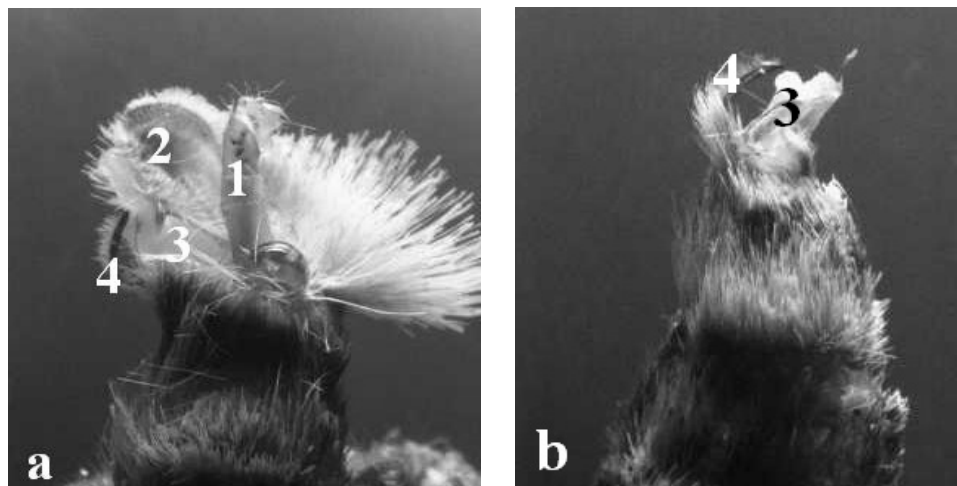


Fig. 2. External reproductive organs of (a) a normal male F₁ hybrid and (b) an abnormal F₁ hybrid (female *Helicoverpa armigera* × male *H. assulta*). 1, aedeagus; 2, valva; 3, tuba analis; 4, uncus.

Table 1. The number of F₁ hybrids from crossing female *Helicoverpa armigera* × male *H. assulta* with different abnormal internal reproductive organs.

Replicates	Total no. adults	No. of adults without reproductive organs	No. of adults with 1–3 copies of undeveloped bursa copulatrix	No. of adults with 1–2 undeveloped testes
1	19	3 (16.8%)	9 (47.4%)	7 (36.8%)
2	37	5 (13.5%)	11 (29.7%)	21 (56.8%)
3	49	11 (22.4%)	9 (18.4%)	29 (59.2%)
Total	105	19 (18.1%)	29 (27.6%)	57 (54.3%)

but only 374 pupated (76%). Among these pupae, 203 individuals were male pupae, 140 abnormal type A pupae and 31 abnormal type B pupae. The percentage of eclosion of the morphologically abnormal types taken together was 51.5%, which was significantly lower than that of the normal F₁ males (95.1%). No significant difference was found in the percentage of eclosion between types A and B abnormal pupae.

The larval development time of F₁ hybrid males was similar to that of males and females of *H. armigera* (table 3a), but abnormal F₁ hybrid larvae took two days longer to develop than the normal hybrids and two days shorter than *H. assulta* males. Similarly, the pupal duration of the abnormal F₁ hybrids was significantly longer than that of the normal F₁ hybrids (table 3b).

The data from the flight ability test are shown in table 4. The F₁ hybrid males achieved much longer flight distances than males of either *H. armigera* or *H. assulta*.

F₁ hybrid of females *H. assulta* × males *H. armigera*

The pupal morphology of the F₁ hybrids derived from crossing female *H. assulta* × male *H. armigera* was similar to that of typical males and females. No morphologically abnormal individuals were found. However, female pupal size was smaller than male pupal size (data not shown). Totals of 83 F₁ hybrid females and 80 males were obtained. The ratio of females to males was 1.04:1. Males and females of the F₁ hybrids were all fertile, and could backcross with their parents and produce offspring.

Sex ratios in hybrids and backcrosses

The sex ratios of parental *H. armigera* (AR) and *H. assulta* (AS) were close to 1:1 (table 5). F₁ hybrids (F₁RS) resulting from the cross of female *H. armigera* × male *H. assulta* comprised 57% normal males and 43% abnormal ones. The

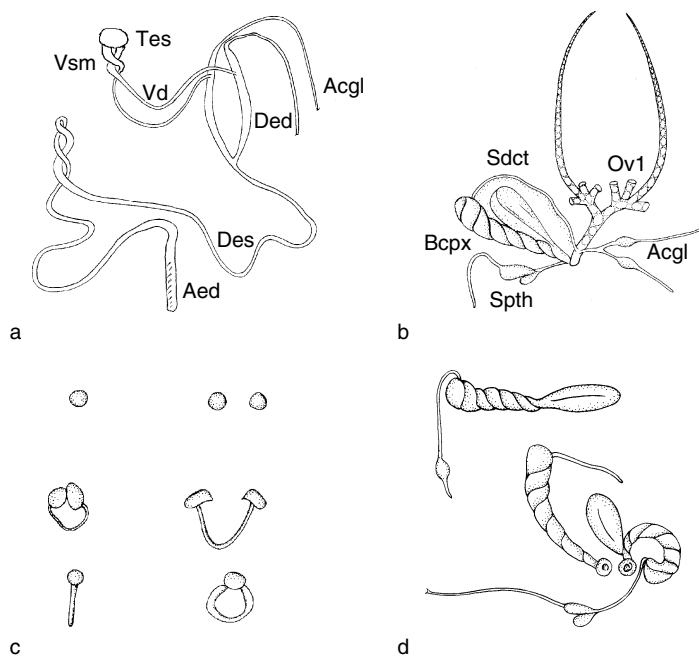


Fig. 3. Normal male (a) and female (b) internal reproductive organs of *Helicoverpa armigera* and abnormal testes (c) and bursa copulatrix (d) of F₁ hybrid (female *H. armigera* × male *H. assulta*). Acgl, paired accessory glands; Aed, aedeagus; Bcpx, bursa copulatrix; Ded, ductus ejaculatorius duplex; Des, ductus ejaculatorius simplex; Ov1, ovariole; Sdct, seminal duct; Spth, spermatheca; Tes, fused testes; Vd, vas deferens; Vsm, seminal vesicles.

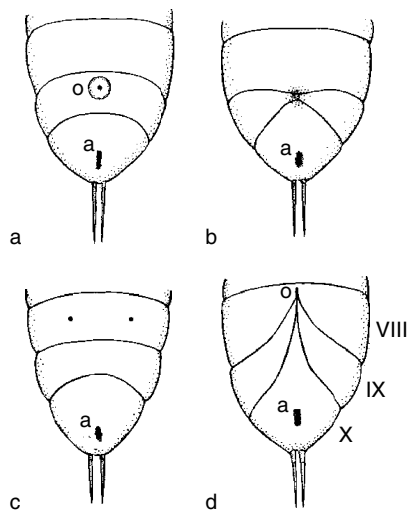


Fig. 4. Normal male pupa (a), type A (b) and type B (c) abnormal pupae in F_1 hybrids (female *Helicoverpa armigera* \times male *H. assulta*) and normal female pupa (d) o, opening of reproductive organ; a, anus; VII, IX and X, the eighth to tenth abdominal segments of the pupa.

backcross of F_1 RS males with *H. armigera* females produced BC_{1a} offspring, comprised mostly of typical males and females, and 11% abnormal individuals. BC_{1a} males mated with females of *H. armigera* produced a backcross BC_{2a} comprised largely of typical males and females and 1.5% abnormal. In the BC_{2b} offspring from female $BC_{1a} \times$ male BC_{1a} and BC_{3a} from female *H. armigera* \times male BC_{2a} , no

abnormal individual was found and the sex ratios were close to 1:1. The sex ratio of the BC_{1b} offspring from female *H. assulta* \times male F_1 RS was 0.89:1; no abnormal individuals were found.

The reverse cross of female *H. assulta* \times male *H. armigera* produced F_1 hybrid (F_1 SR) males and females. Individuals of F_1 hybrid F_1 SR could be self-mated to produce an F_2 generation. The sex ratios of F_1 SR and F_2 were close to 1:1. In BC_{1c} (female AR \times male F_1 SR), the sex ratio was distorted (0.53:1) and there were 12% abnormal individuals. There were also 3.8% abnormal individuals in BC_{1d} (female AS \times male F_1 SR). No abnormal offspring were found in BC_{1e} (female F_1 SR \times male AR) and BC_{1f} (female F_1 SR \times male AS). However, the sex ratio of BC_{1e} was 0.58:1, which was significantly different from 1:1 ($\chi^2 = 5.762$; $df = 1$; $P = 0.016$).

Discussion

The viable and fertile F_1 hybrids from the reciprocal crosses between *H. armigera* and *H. assulta* indicated that the barriers of reproductive isolation between the two *Helicoverpa* species existing in nature could be broken under experimental conditions (Wang & Dong, 2001). Natural hybrids between these species have never been found in the field. Prezygotic isolation mechanisms probably play a potential role in preventing gene flow between two species (Wu *et al.*, 1990; Park *et al.*, 1996; Liu *et al.*, 1994; Wu *et al.*, 1997; Chen, 1999). The host-plant range of the two species is quite different. *Helicoverpa armigera* is a typical generalist and *H. assulta* a specialist. The same two main sex pheromone components are used by the two species, but their ratio ((Z)-11-hexadecenal to (Z)-9-hexadecenal) is about opposite (97:3

Table 2. The numbers of larvae and pupae, percent pupation and eclosion and sex ratio of F_1 hybrids from female *Helicoverpa armigera* \times male *H. assulta* in three replicate crossing events.

	No. of Larvae	% Pupation	No. of pupae			% Male eclosion	% Type A and type B eclosion	Male: (type A + type B)	χ^2 ; P
			Male	Abnormal type A	Abnormal type B				
1	124	67.7	46	30	8	89.1	52.6	1: 0.83	0.762; 0.383
2	170	70	61	47	11	98.4	51.7	1: 0.95	0.076; 0.783
3	198	86.4	96	63	12	92.7	50.7	1: 0.78	2.58; 0.108
Total	492	76.2	203	140	31	95.1	51.5	1: 0.84	2.78; 0.098

Table 3. Development times of larvae (a) and pupae (b) (means \pm SEM) of *Helicoverpa armigera* (AR), *H. assulta* (AS), and the F_1 hybrid F_1 RS. Numbers in brackets represent the sample size.

(a)	Duration of larval stage (days)			t-value; P
	Female	Male	Abnormal	
AR	15.6 \pm 0.29 (14)	15.1 \pm 0.18 (29)	–	1.419; 0.164
F_1 RS	–	15.2 \pm 0.29 (46)	17.7 \pm 0.31 (38)	5.713; 0.000
AS	20.1 \pm 0.41 (19)	19.8 \pm 0.41 (17)	–	0.498; 0.622
(b)	Duration of pupal stage (days)			t-value; P
	Female	Male	Abnormal	
AR	12.9 \pm 0.40 (14)	14.7 \pm 0.14 (29)	–	4.196; 0.001
F_1 RS	–	11.4 \pm 0.13 (41)	14.2 \pm 0.26 (32)	9.636; 0.000
AS	11.7 \pm 0.45 (11)	12.8 \pm 0.60 (13)	–	1.347; 0.192

Table 4. Parameters for flight ability of males of *Helicoverpa armigera* (AR), *H. assulta* (AS) and the F₁ hybrid (F₁RS) (means \pm SEM).

Insect lines	<i>n</i>	Total times (24 h)	Total circles (24 h)	Distance (km)	Speed (km h ⁻¹)	Flight time (h)
AR	8	22 \pm 7.43a	7527 \pm 4020a	7.53 \pm 4.02a	2.19 \pm 0.16a	2.85 \pm 1.42a
F ₁ RS	11	48.9 \pm 9.02b	48320 \pm 25100b	48.3 \pm 25.1b	2.76 \pm 0.33a	8.81 \pm 1.55b
AS	8	29 \pm 9.53a	14160 \pm 4600a	14.2 \pm 4.59a	2.40 \pm 0.16a	5.71 \pm 1.76ab

Means followed the same letter within a column were not significantly different ($P = 0.05$).

Table 5. Ratio of female, male and abnormal pupae of *Helicoverpa armigera* (AR) and *H. assulta* (AS), their hybrids and backcross offspring.

Cross (female \times male)	No. of females (F)	No. of males (M)	No. of abnormal individuals (AN)	Total	Ratio (F:M:AN)	Expected ratio (F:M:AN)	χ^2 ; df; <i>P</i>
AR \times AR = AR	351	364	–	715	0.96:1:0	1:1:0	0.236; 1; 0.267
AS \times AS = AS	235	205	–	440	1.14:1:0	1:1:0	2.05; 1; 0.153
AR \times AS = F ₁ RS	–	190	145	335	0.00:1:0.76	0:1:1	6.05; 1; 0.014
AS \times AR = F ₁ SR	83	80	–	163	1.04:1:0	1:1:0	0.055; 1; 0.814
F ₁ SR \times F ₁ SR = F ₂	66	75	–	141	0.88:1:0	1:1:0	0.574; 1; 0.448
AR \times F ₁ RS = BC ₁ a	113	218	41	372	0.50:1:0.19	1:2:1	38.38; 2; 0.000
AR \times BC ₁ a = BC ₂ a	119	144	4	267	0.83:1:0.03	3:4:1	32.15; 2; 0.000
BC ₁ a \times BC ₁ a = BC ₂ b	194	221	–	415	0.80:1:0	1:1:0	1.175; 1; 0.185
AR \times BC ₂ a = BC ₃	54	69	–	123	0.78:1:0	1:1:0	1.829; 1; 0.176
AS \times F ₁ RS = BC ₁ b	112	127	–	239	0.89:1:0	1:1:0	0.941; 1; 0.332
AR \times F ₁ SR = BC ₁ c	126	240	51	417	0.53:1:0.21	1:2:1	36.50; 2; 0.000
AS \times F ₁ SR = BC ₁ d	53	48	4	105	1.04:1:0.08*	1:1:0	0.248; 1; 0.619*
F ₁ SR \times AR = BC ₁ e	31	53	–	84	0.58:1:0	1:1:0	5.762; 1; 0.016
F ₁ SR \times AS = BC ₁ f	113	126	–	239	0.90:1:0	1:1:0	0.707; 1; 0.400

* The abnormal specimens in BC₁d were not subjected to Chi-square test.

in *H. armigera* and 7:93 in *H. assulta*). The female calling period of *H. armigera* was found to occur from the fifth hour to the seventh hour in the scotophase, but that of *H. assulta* was from the third hour to the fifth hour indicating a small degree of overlap between the two species (unpublished data).

As in all the other Lepidoptera, the female of *Helicoverpa* species is the heterogametic sex and the system of sex determination is ZW/ZZ. So in theory, a *H. armigera* female crossed with a *H. assulta* male produces a heterogametic female hybrid (ZW) and a homogametic male hybrid (ZZ). In fact, the F₁ hybrid from this cross consisted of normal fertile males and abnormal sterile individuals. According to Haldanes rule, it is assumed that the sterile abnormal F₁-individuals have a female genotype ZW. However, the F₁ abnormal individuals were always fewer in number than normal males of F₁. Some degree of lethality was expected in ZW genotype hybrids. This prediction was confirmed by the higher mortality in the pupal stage.

Large Z effects resulting in hybrid sterility and inviability seem quite common in Lepidoptera (Turelli & Orr, 2000; Jiggins *et al.*, 2001; Presgraves, 2002). In the gypsy moth, *Lymantria dispar* (Linnaeus) (Lepidoptera: Lymantriidae), intersexuals or sterile females were produced from crossings between two geographically separated populations. The reason for this was that the Z chromosome carried a male sex determining factor from a strong *L. dispar* race that was dominant over a female determinant from a weak *L. dispar* race (Downes, 1959). The sterility of male offspring from interspecific hybridization between female *Heliothis subflexa* \times male *H. virescens* was shown to be caused by

abnormal sperm (Laster, 1972; Proshold & LaChance, 1974; Richard *et al.*, 1974; Goodpasture *et al.*, 1980a,b; LaChance & Karpenko, 1983; LaChance, 1984; Miller *et al.*, 1986; Miller & Miller, 1996), which was evidently caused by cytoplasmic factors conflicting with the Z chromosome (Laurie, 1997). In races of *Heliconius melpomene* (Linnaeus) (Lepidoptera: Nymphalidae), female F₁ hybrids are sterile when a male from French Guiana is crossed with a female from Panama, but fertile in the reciprocal cross; male F₁s are fertile in both directions. Backcrosses and linkage analysis show that sterility results from an interaction between (a) gene(s) on the Z chromosome of the Guiana race with autosomal factors in the genome of Panama race (Jiggins *et al.*, 2001).

The interspecific hybridization experiments of *H. armigera* and *H. assulta* also reveal differences in reciprocal crosses (asymmetry). The abnormal or sterile moths derived from crosses may result from either Z-linked or cytoplasmic incompatibility. Turelli & Orr (2000) demonstrated that maternal factors had no effects on the sterility of female-heterogametic species. The sterility found in F₁ hybrids (female *H. armigera* \times male *H. assulta*) and both backcross offspring, BC₁a (AR \times F₁RS) and BC₁c (AR \times F₁SR) indicated that the abnormalities resulted from the interactions between the Z-chromosome from *H. assulta* and the W-chromosome from *H. armigera* or between the Z-chromosome and autosomes. However, there were four abnormal individuals in the BC₁d backcross (AS \times F₁SR). This finding is not in agreement with the incompatibility of the Z-chromosome from *H. assulta* with the W-chromosome from *H. armigera* since their W-chromosome was inherited from *H. assulta*. This suggests that the sterility resulted from the incompatibility

of the Z-chromosome with the autosomal genes. The occurrence of fewer abnormal F₁ hybrids than normal male F₁ hybrids suggested that inviability resulted from interspecific hybridization. Sex bias with a lower proportion of females in BC₁e offspring (F₁SR × AR) indicated that inviability existed in this cross. Maternal-zygotic incompatibilities and dominance could result in inviability of female-heterogametic species (Turelli & Orr, 2000). Abnormal F₁ hybrids have W/cytoplasm from *H. armigera* and Z-chromosome from *H. assulta*, while female offspring of the BC₁e backcross (F₁SR × AR) have W/cytoplasm from *H. assulta* and Z-chromosome from *H. armigera*. These indicated that maternal-zygotic incompatibilities resulted in hybrid inviability. Therefore, the interaction of the Z-chromosome from *H. assulta* with autosomes from *H. armigera* might result in different degrees of sterility and maternal-zygotic incompatibilities might contribute to sex bias.

In insects of economic importance, verification of the physiological potential for hybrid production and viability of offspring has special implications. If hybrids are fertile or partially fertile, introduction of genes from different species through hybridization might result in insects with new traits. Proshold (1983) reported that male sterility factors were successfully infused in the native *H. virescens* population through large-scale release of hybrid females on St Croix, US Virgin Islands in 1979 and 1980. When backcrossed with the native males of *H. virescens*, the hybrid females produced sterile males, thus realizing control of *H. virescens*. Also in this study abnormal or sterile offspring were produced by interspecific hybridization. The backcross of female *H. armigera* with the F₁ males derived from female *H. armigera* and male *H. assulta* produced offspring with a biased sex ratio and 43% of sterility. Such an approach has some potential in *H. armigera* control. These tests have shown that the male hybrid has a better flight ability than the typical male *H. armigera*. However, introduction of genetic variability through hybridization might result in colonization of new habitats by this polyphagous insect. Whether interspecific hybridization could be used for genetic control of *H. armigera* in an integrated pest management programme deserves further investigation.

Acknowledgements

The authors wish to thank H.X. Han for morphological determination of moths, L. Feng for insect rearing, S.-Y. Ma for drawing the pictures, and Professor D.-F. Cheng, Dr Z. Tian and Dr X.-F. Jiang for their help in the flight experiment. This work was supported by the Chinese Academy of Sciences (grant no. KSCX2-SW-105), a special fund for Major State Basic Research Project of China (grant no. 2000016208), and the National Natural Science Foundation of China (grant no. 30330100).

References

- Chen, Y.X. (1999) *Fauna Sinica Vol. 16: Insecta, Lepidoptera, Noctuidae*. Beijing, Science Press. pp. 145–147 (in Chinese).
- Cheng, D.F., Tian, Z., Li, H.M., Sun, J.R. & Chen, J.L. (2002) Influence of temperature and humidity on the flight capacity of *Sitobion avenae*. *Acta Entomologica Sinica* **45**, 80–85.
- Coyne, J.A. & Orr, H.A. (1989) Patterns of speciation in *Drosophila*. *Evolution* **43**, 362–381.
- Degrugillier, M.E. & Newman, S.M. Jr. (1993) Hereditary viruses of *Heliothis*? Chromatin-associated virus-like particles in testes of six species of *Heliothis* and *Helicoverpa*, F₁, and backcross males. *Journal of Invertebrate Pathology* **61**, 147–155.
- Downes, J.A. (1959) The gypsy moth and some possibilities of the control of insects by genetical means. *Canadian Entomologist* **91**, 661–664.
- Fitt, G.P. (1989) The ecology of *Heliothis* spp. in relation to agroecosystems. *Annual Review of Entomology* **34**, 17–52.
- Goodpasture, C., LaChance, L.E. & Richard, R.D. (1980a) Persistence of abnormal spermiogenesis in the backcross generations of interspecific hybrids between *Heliothis virescens* × *H. subflexa*. *Annals of the Entomological Society of America* **73**, 397–403.
- Goodpasture, C., Richard, R.D., Martin, D. & Laster, M. (1980b) Sperm cell abnormalities in progeny from interspecific crosses between *Heliothis virescens* and *H. subflexa*. *Annals of the Entomological Society of America* **73**, 529–532.
- Gruha, J.W. & Taylor, O.R. (1980) Some characteristics of hybrids derived from the sulfur butterflies, *Colias eurytheme* and *C. philodice*: phenotypic effects of the X-chromosome. *Evolution* **34**, 673–687.
- Hardwick, D.F. (1965) The corn earworm complex. *Memoirs of the Entomological Society of Canada* **40**, 14–19.
- Jiggins, C.D., Linares, M., Naisbit, R.E., Salazar, C., Yang, Z.H. & Mallet, J. (2001) Sex-linked hybrid sterility in a butterfly. *Evolution* **55**, 1631–1638.
- Knipling, E.F. (1960) Use of insects for their own destruction. *Journal of Economic Entomology* **53**, 415–420.
- Krueger, C.M., Degrugillier, M.E. & Narang, S.K. (1993) Size difference among 16S rRNA genes from endosymbiotic bacteria found in testes of *Heliothis virescens*, *H. subflexa* (Lepidoptera: Noctuidae), and backcross sterile male moths. *Florida Entomologist* **76**, 382–383.
- LaChance, L.E. (1984) Hybrid sterility: eupyrene sperm production and abnormalities in the backcross generations of interspecific hybrids between *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **77**, 93–101.
- LaChance, L.E. & Karpenko, C.P. (1983) Hybrid sterility in *Heliothis subflexa* × *H. virescens* (Lepidoptera: Noctuidae) crosses: expression after injection with antiviral agents, heat shocks, and rearing at extreme temperatures. *Annals of the Entomological Society of America* **76**, 104–109.
- Laster, M.L. (1972) Interspecific hybridization of *Heliothis virescens* and *H. subflexa*. *Environmental Entomology* **6**, 682–687.
- Laster, M.L. & Hardee, D.D. (1995) Inter-mating compatibility between North American *Helicoverpa zea* and *Heliothis armigera* (Lepidoptera: Noctuidae) from Russia. *Journal of Economic Entomology* **88**, 77–80.
- Laster, M.L. & Sheng, C.F. (1995) Search for hybrid sterility for *Helicoverpa zea* in crosses between the North American *H. zea* and *H. armigera* (Lepidoptera: Noctuidae) from China. *Journal of Economic Entomology* **88**, 1288–1291.
- Laster, M.L., King, E.G. & Furr, R.E. (1988) Interspecific hybridization of *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae) from Argentina. *Environmental Entomology* **17**, 1016–1018.
- Laurie, C.C. (1997) The weaker sex is heterogametic: 75 years of Haldane's rule. *Genetics* **147**, 937–951.
- Liu, M.Y., Cai, J.P. & Tian, Y. (1994) Sex pheromone components of the oriental tobacco budworm, *Helicoverpa assulta*

- Guenée: identification and field trials. *Entomologica Sinica* **1**, 77–5.
- Miller, S.G. & Miller, R.D.** (1996) Infectious enterococcus from *Heliothis virescens* × *H. subflexa* backcross hybrids (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **89**, 420–427.
- Miller, S.G., Huettel, M.D., Davis, M.T.B., Weber, E.H. & Weber, L.A.** (1986) Male sterility in *Heliothis virescens* × *H. subflexa* backcross hybrids, evidence for abnormal mitochondrial transcripts in testes. *Molecular Genetics and Genomics* **203**, 451–461.
- Park, K.C., Cork, A. & Boo, K.S.** (1996) Intrapopulation changes in sex pheromone composition during scotophase in oriental tobacco budworm, *Helicoverpa assulta* (Guenée) (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* **22**, 1201–1210.
- Presgraves, D.C.** (2002) Patterns of postzygotic isolation in Lepidoptera. *Evolution* **56**, 1168–1183.
- Proshold, F.I.** (1983) Release of backcross insects on St. Croix, U.S. Virgin Islands, to suppress the tobacco budworm (Lepidoptera: Noctuidae): infusion of sterility into a native population. *Journal of Economic Entomology* **76**, 1353–1359.
- Proshold, F.I. & LaChance, L.E.** (1974) Analysis of sterility in hybrids from interspecific crosses between *Heliothis virescens* and *H. subflexa*. *Annals of the Entomological Society of America* **67**, 445–449.
- Richard, R.D., LaChance, L.E. & Proshold, F.I.** (1974) An ultrastructural study of sperm in sterile hybrids from crosses of *Heliothis virescens* and *H. subflexa*. *Annals of the Entomological Society of America* **67**, 35–39.
- SPSS** (2001) *SPSS 11.01 for Windows*. SPSS Inc, Chicago, Illinois.
- Turelli, M. & Orr, H.A.** (2000) Dominance, epistasis and the genetics of postzygotic isolation. *Genetics* **154**, 1663–1679.
- Wang, C.Z. & Dong, J.F.** (2001) Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera: Noctuidae). *Chinese Science Bulletin* **46**, 489–491.
- Wu, D.M., Yan, Y.H. & Cui, J.R.** (1997) Sex pheromone components of *Helicoverpa armigera*: chemical analysis and field tests. *Entomologica Sinica* **4**, 350–56.
- Wu, K.J. & Gong, P.Y.** (1997) A new and practical artificial diet for the cotton bollworm. *Entomologica Sinica* **4**, 277–282.
- Wu, W.Q., Tang, X.H., Xu, S.F. & Du, J.W.** (1990) Diel periodicity of female calling activity and sex pheromone production in *Heliothis armigera* (Lepidoptera: Noctuidae). *Contributions from Shanghai Institute of Entomology* **10**, 57–62.

(Accepted 18 April 2005)

© CAB International, 2005