

## Hybridization between *Helicoverpa armigera* and *Helicoverpa assulta* (Lepidoptera: Noctuidae): development and morphological characterization of F<sub>1</sub> hybrids

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### 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 F<sub>1</sub> 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

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## 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 F<sub>1</sub> females and sterile males. Backcrosses of F<sub>1</sub> 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 F<sub>1</sub> hybrids of female *H. assulta* and males *H. armigera* are fertile with a 1:1 sex ratio. The F<sub>1</sub> hybrids derived from female *H. armigera* × male *H. assulta* yielded fertile males but no females (Wang & Dong, 2001). The latter F<sub>1</sub> hybrid males showed heterosis in pupal weight and adult activity, and the backcross line of these F<sub>1</sub> 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 F<sub>1</sub> hybrids between *H. armigera* and *H. assulta*. Abnormal individuals were identified in the F<sub>1</sub> 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 F<sub>1</sub> hybrids. The various backcrosses were designed to test the fertility of F<sub>1</sub> hybrids.

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

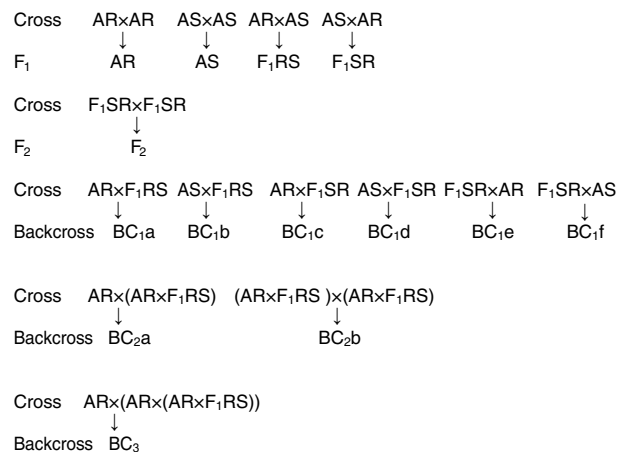


Fig. 1. Female and male crossing scheme employed to produce hybrids between *Helicoverpa armigera* (AR) and *H. assulta* (AS) and backcrosses.

collected daily and hatched larvae of F<sub>1</sub> generations were reared under the conditions described above.

For the experimental F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> hybrids. The Chi-square test was used to compare ratios of female, male, and abnormal individuals with expected values.

### Results

#### The F<sub>1</sub> hybrid of female *H. armigera* × male *H. assulta*

Adults of the F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> hybrid generation were obtained (table 2),

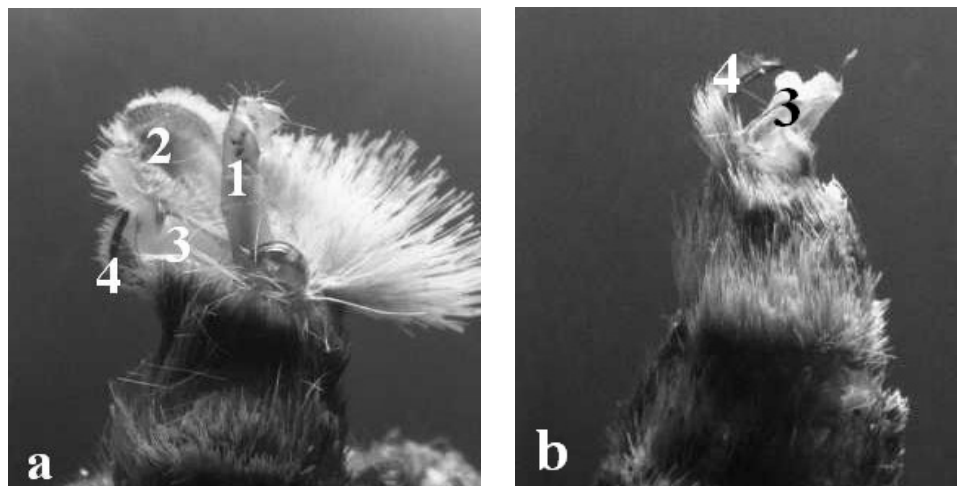


Fig. 2. External reproductive organs of (a) a normal male F<sub>1</sub> hybrid and (b) an abnormal F<sub>1</sub> hybrid (female *Helicoverpa armigera* × male *H. assulta*). 1, aedeagus; 2, valva; 3, tuba analis; 4, uncus.

Table 1. The number of F<sub>1</sub> 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 F<sub>1</sub> 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 F<sub>1</sub> hybrid males was similar to that of males and females of *H. armigera* (table 3a), but abnormal F<sub>1</sub> 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 F<sub>1</sub> hybrids was significantly longer than that of the normal F<sub>1</sub> hybrids (table 3b).

The data from the flight ability test are shown in table 4. The F<sub>1</sub> hybrid males achieved much longer flight distances than males of either *H. armigera* or *H. assulta*.

#### F<sub>1</sub> hybrid of females *H. assulta* × males *H. armigera*

The pupal morphology of the F<sub>1</sub> 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 F<sub>1</sub> hybrid females and 80 males were obtained. The ratio of females to males was 1.04:1. Males and females of the F<sub>1</sub> 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). F<sub>1</sub> hybrids (F<sub>1</sub>RS) resulting from the cross of female *H. armigera* × male *H. assulta* comprised 57% normal males and 43% abnormal ones. The

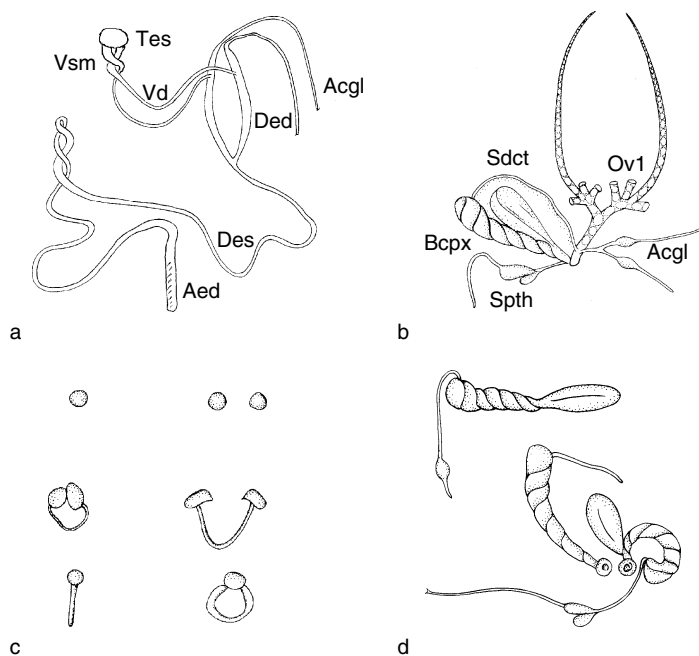


Fig. 3. Normal male (a) and female (b) internal reproductive organs of *Helicoverpa armigera* and abnormal testes (c) and bursa copulatrix (d) of F<sub>1</sub> hybrid (female *H. armigera* × male *H. assulta*). Acgl, paired accessory glands; Aed, aedeagus; Bcpx, bursa copulatrix; Ded, ductus ejaculatorius duplex; Des, ductus ejaculatorius simplex; Ov1, ovariole; Sdct, seminal duct; Spth, spermatheca; Tes, fused testes; Vd, vas deferens; Vsm, seminal vesicles.

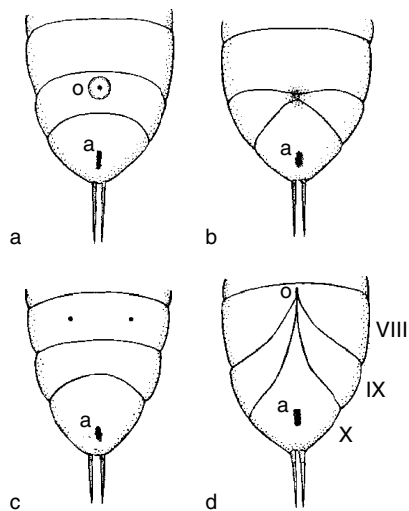


Fig. 4. Normal male pupa (a), type A (b) and type B (c) abnormal pupae in  $F_1$  hybrids (female *Helicoverpa armigera*  $\times$  male *H. assulta*) and normal female pupa (d) o, opening of reproductive organ; a, anus; VII, IX and X, the eighth to tenth abdominal segments of the pupa.

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)	$\chi^2$ ; 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 F<sub>1</sub> hybrid (F<sub>1</sub>RS) (means  $\pm$  SEM).

Insect lines	<i>n</i>	Total times (24 h)	Total circles (24 h)	Distance (km)	Speed (km h <sup>-1</sup> )	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 <sub>1</sub> 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)	$\chi^2$ ; 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 <sub>1</sub> RS	–	190	145	335	0.00:1:0.76	0:1:1	6.05; 1; 0.014
AS $\times$ AR = F <sub>1</sub> SR	83	80	–	163	1.04:1:0	1:1:0	0.055; 1; 0.814
F <sub>1</sub> SR $\times$ F <sub>1</sub> SR = F <sub>2</sub>	66	75	–	141	0.88:1:0	1:1:0	0.574; 1; 0.448
AR $\times$ F <sub>1</sub> RS = BC <sub>1</sub> a	113	218	41	372	0.50:1:0.19	1:2:1	38.38; 2; 0.000
AR $\times$ BC <sub>1</sub> a = BC <sub>2</sub> a	119	144	4	267	0.83:1:0.03	3:4:1	32.15; 2; 0.000
BC <sub>1</sub> a $\times$ BC <sub>1</sub> a = BC <sub>2</sub> b	194	221	–	415	0.80:1:0	1:1:0	1.175; 1; 0.185
AR $\times$ BC <sub>2</sub> a = BC <sub>3</sub>	54	69	–	123	0.78:1:0	1:1:0	1.829; 1; 0.176
AS $\times$ F <sub>1</sub> RS = BC <sub>1</sub> b	112	127	–	239	0.89:1:0	1:1:0	0.941; 1; 0.332
AR $\times$ F <sub>1</sub> SR = BC <sub>1</sub> c	126	240	51	417	0.53:1:0.21	1:2:1	36.50; 2; 0.000
AS $\times$ F <sub>1</sub> SR = BC <sub>1</sub> d	53	48	4	105	1.04:1:0.08*	1:1:0	0.248; 1; 0.619*
F <sub>1</sub> SR $\times$ AR = BC <sub>1</sub> e	31	53	–	84	0.58:1:0	1:1:0	5.762; 1; 0.016
F <sub>1</sub> SR $\times$ AS = BC <sub>1</sub> f	113	126	–	239	0.90:1:0	1:1:0	0.707; 1; 0.400

\* The abnormal specimens in BC<sub>1</sub>d 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 F<sub>1</sub> hybrid from this cross consisted of normal fertile males and abnormal sterile individuals. According to Haldanes rule, it is assumed that the sterile abnormal F<sub>1</sub>-individuals have a female genotype ZW. However, the F<sub>1</sub> abnormal individuals were always fewer in number than normal males of F<sub>1</sub>. 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 F<sub>1</sub> hybrids are sterile when a male from French Guiana is crossed with a female from Panama, but fertile in the reciprocal cross; male F<sub>1</sub>s 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 F<sub>1</sub> hybrids (female *H. armigera*  $\times$  male *H. assulta*) and both backcross offspring, BC<sub>1</sub>a (AR  $\times$  F<sub>1</sub>RS) and BC<sub>1</sub>c (AR  $\times$  F<sub>1</sub>SR) 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 BC<sub>1</sub>d backcross (AS  $\times$  F<sub>1</sub>SR). 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 F<sub>1</sub> hybrids than normal male F<sub>1</sub> hybrids suggested that inviability resulted from interspecific hybridization. Sex bias with a lower proportion of females in BC<sub>1</sub>e offspring (F<sub>1</sub>SR × 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 F<sub>1</sub> hybrids have W/cytoplasm from *H. armigera* and Z-chromosome from *H. assulta*, while female offspring of the BC<sub>1</sub>e backcross (F<sub>1</sub>SR × 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 F<sub>1</sub> 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.

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