Interspecific hybridization of *Helicoverpa armigera* and *H. assulta* (Lepidoptera: Noctuidae)

WANG Chenzhu & DONG Junfeng

State Key Laboratory of Integrated Management of Pest and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China
Correspondence should be addressed to Wang Chenzhu (e-mail: czwang@panda.ioz.ac.cn)

Abstract **Reciprocal hybridization between *Helicoverpa armigera* (Hübner) and *H. assulta* Guenee followed by backcrossing of the hybrids (F_1) with *H. armigera* produced backcross (BC) lines consisting of fertile females and males. The F_1 of *H. armigera* female and *H. assulta* male had only male, no female sex. In this case Haldane's rule applies, and therefore it is proved that the sex chromosomes of *Helicoverpa* species are of ZW type, and the female is the heterozygous sex. This hybrid also showed significant heterosis with the heaviest pupal weight, and when it was backcrossed with *H. armigera* female, the sex ratio of the BC offspring was distorted as 1∶4. The potential utilization of this hybrid in genetic controlling *H. armigera* was finally discussed.**

Keywords: *Helicoverpa armigera*, *H. assulta*, interspecific hybridization.

The sibling species *Helicoverpa armigera* (Hübner) and *H. assulta* are serious crop pests in China[1]. They have the similar appearance and feeding behavior, but their host-plant ranges are quite different. *H. armigera* is a typical polyphagous species, its host plant range includes at least 60 crop species such as cotton, corn, wheat, soybean, tobacco and tomato and 67 wild plant species from about 30 plant families including Malvaceae, Solanaceae, Gramineae, Leguminosae, etc.; *H. assulta* is an oligophagous species with a relatively narrow host plant range, and mainly feeds on plant species in Solanaceae such as tobacco, hot pepper, and several *Physalis* species[2]. *H. armigera* has developed resistance to a wide range of insecticides, and causes significant damage to many important agricultural crops in China each year; for example, the outbreak of this pest in 1992 resulted in a loss of lint cotton about 30%[3]. Therefore, new effective control methods are urgently needed. Research on hybridization of the two related species may not only promote our understanding of their host-plant preferences, but also help us to find new insect lines for pest genetic control.

Genetic control is one of the most important pest control methods. The basis of this method is the release of laboratory-reared individuals that carry some specific genetic factors into a natural population in order to manipulate the pest population. The sterile insect release technique in which males are sterilized by irradiation, has been first used to control the screwworm (*Cochliomyia hominivorax*) successfully, and then has been applied to other pest insect species, such as the mediterranean fruit fly (*Ceratitis capitata*), tsetse flies (*Glossina palpalis* and *G. morsitans*), mosquitoes (*Anopheles albimanus*), and codling moth (*Cydia pomonella*)[4]. In 1960, Knipling[5] was the first to suggest that the sterile hybrids of two intercrossing related species might be possible for genetic control of some insects. Laster[6] reported that *Heliothis virescens* and *H. subflexa* were successfully hybridized, and discovered that the hybrid and backcross progeny had inherited male sterility. These laboratory-reared backcross insects were released on St. Croix, U. S. Virgin Islands to suppress *H. virescens*, and the investigations indicated that the sterility trait was infused into the native population during and after the release of backcross insects, and proved the feasibility of managing *H. virescens* population by releasing backcross insects with inherited male sterility trait[7]. In order to search similar type sterility for *Helicoverpa zea*, interspecific crosses between *H. zea* and *H. armigera* and backcrosses were evaluated, but sterility was not detected, all crosses mated and produced offspring[8]. Concerning interspecific crosses between *H. armigera* and *H. assulta*, there was an only report that two species could not mate with each other and no hybrid was produced, but apparently the sample size in the research was too small[9]. The aim of present study is to clarify the possibility of interspecific hybridization of these two species and evaluate the sterility of possible hybrids.

1 Materials and methods

(ⅰ) Insect colonies and environmental conditions. *H. armigera* and *H. assulta* was collected in the cotton field of Anyang and the tobacco field of Xuchang, Henan Province respectively. Colonies were maintained in the rearing room at (27±1)℃ with a light regime of L15: D9. Moths were fed with 10% honey solution and mated in a group of about 20 pairs in a 40 cm×25 cm×18 cm cage with a removable mesh cloth top for egg collection. The eggs were hatched in a jar, then 3 neonate larvae were transferred to the artificial diet in each 25 mL glass tube. In the 3rd instar and later on, larvae were reared singly in glass tubes. The composition of artificial diets for larvae of *H. armigera* and *H. assulta* was cooked soybean powder (105 g), yeast powder (32 g), wheat germ (50 g), ascorbic acid (3.3 g), methyl parahydrobenzoate (2 g), sorbic acid (1 g), agar (14.5 g) and distilled water (640 mL). The preparatory procedure of the diet was as the method of Wu[10]. When the larvae pupated, they were separated by sex and placed on autoclaved moist soil in jars, and held for adult emergence. About a thousand individuals of each species in the uniform stage were reared in each generation.

(ii) Test methods. The studying scheme for cross and backcross of two species is shown in fig. 1. Pupae of each species, hybrid or backcross offspring were separated by sex and were held for adult emergence. After emergence, virgin male and female moths of *H. armigera* were paired with virgin female and male moths of *H. assulta* in the cage, and 73 and 55 pairs of insects were used in combinations of *H. armigera* ♀ vs. *H. assulta* ♂ and *H. assulta* ♀ vs. *H. armigera* ♂ respectively. Thirty pairs of each parent species were used as control. The eggs oviposited on the inside of cage top were collected by day, the hatching rate of eggs laid in oviposition peak was counted. When adult moth died, the female was dissected to determine mating status according to the presence or absence of spermatophores. Newly hatched larvae of different species and hybrids were reared individually in glass tubes on artificial diet. Upon pupation, pupae were sexed and pupal weight were calculated. The test ran three replications.

The hybrids (SR and RS) produced in reciprocal hybridization of two species were backcrossed with *H. armigera* male or female as the scheme shown in fig. 1, 15 pairs of moths were used in each of three backcrosses. Sex ratio and pupal weight of backcross lines were also counted as above with three replications.

Data collected were subjected to analyses of variance (ANOVA) and Duncan's multiple range tests (P = 0.05) for differences among species, hybrids, and backcross offspring.

Parents	S × R	R × S	R × R	S × S
	↓	↓	↓	↓
F_1	SR	RS	R	S
	SR × R	R × SR	R × RS	
	↓	↓	↓	
BF	BC1	BC2	BC3	

Fig. 1. Study scheme of reciprocal cross of *Helicoverpa armigera* (R) and *H. assulta* (S), and backcross of hybrids (SR and RS) and *H. armigera*.

2 Results and analyses

The female moths of *H. armigera* and *H. assulta* could produce eggs no matter whether they were mated or not, but unfertilized eggs laid by unmated females could not hatch, only fertilized eggs laid by mated females could develop. In combinations of *H. armigera* ♀ vs. *H. assulta* ♂ and *H. assulta* ♀ vs. *H. armigera* ♂, the hatching rates of offspring were 8.18% and 6.13% respectively, which means that only less than 10% of the eggs were fertilized. Further dissections of females to determine the presence or absence of spermatophores showed that parent females mated were only 7.14% and 8.82%. More than 85% of the eggs laid by *H. armigera* colonies or *H. assulta* colonies were fertitilized. The sex ratio and pupal weight of each cross or backcross progeny are presented in table 1. The sex ratios of *H. armigera* and *H. assulta* were both about 1 ∶ 1, the average weights of female pupae of both species had no difference, but the average pupal weight of male *H. armigera* was remarkably heavier than that of male *H. assulta*. Hybrids (F_1) of two reciprocal crosses had different sex ratios. The F_1 of (*H. assulta* ♀ × *H. armigera* ♂) had about the same number of male and female offspring (SR), and their average pupal weight was just like their parents. Otherwise, the F_1 of (*H. armigera* ♀ × *H. assulta* ♂) produced only male offspring (RS) with a significantly heavy average pupal weight (357 mg), but the female sex was absent.

Table 1 Sex ratio and pupal weight of *Helicoverpa armigera* (R) and *H. assulta* (S), hybrids (SR and RS), and backcross progenies (BC1, BC2 and BC3)[a)]

Cross female × male	Sex ratio ± SEM female/(female+male)	Female pupal weight ± SEM/mg	Male pupal weight ± SEM /mg
R×R=P1	0.48±0.06 a	0.3032±0.0054 ab	0.3025±0.0059 b
S×S=P2	0.43±0.06 a	0.2883±0.0061 ab	0.2820±0.0086 c
S×R=SR	0.53±0.06 a	0.2841±0.0062 b	0.2779±0.0060 c
R×S=RS	0 * c		0.3569±0.0072 a
SR×R=BC1	0.44±0.06 a	0.3057±0.0080 a	0.2977±0.0073 bc
R×SR=BC2	0.44±0.04 a	0.2974±0.0066 ab	0.3083±0.0065 b
R×RS=BC3	0.20±0.04 b	0.3074±0.0068 a	0.3058±0.0063 b

a) For sex ratio data, n = 3; for pupal weight, n = 30. Means followed by the same letter within a column were not significantly different (P = 0.05; Duncan's multiple range test). * The total number of the hybrid RS individuals obtained in the experiment were 261, no one was female.

Backcross lines of (SR ♀ × *H. armigera* ♂) and (*H. armigera* ♀ × SR ♂) both had a sex ratio of 1 ∶ 1, while the backcross line of (*H. armigera* ♀ × RS ♂) had a distorted sex ratio (1 ∶ 4). The average pupal weights of backcross lines were similar to that of *H. armigera*.

3 Discussion

In genus *Helicoverpa* and *Heliothis*, interspecific hybridization studied could be concluded into the following categories: i) Two species could mate with each other, but had no offspring. Hybridizations of *Helicoverpa punctigera* with *H. zea* and *H. armigera* belonged to this category[11]. ii) Two species could produce hybrids, but one sex was sterile. The cross between *Heliothis subflexa* female and *H. virescens* male produced fertile female and sterile male, and male sterile trait is inherited in the following backcross generations[6]. iii) Two species could produce hybrids, and hybrids were all fertile. The hybridation of *H. armigera* and *H. zea* could produce fertile male and female offsprings, even though the hybridization rate is quite low[8,11,12]. Our study indicated that hybridization of *H. armigera* and *H. assulta* belonged to the third category. We discovered that the hybrids of *H. armigera* female and *H. assulta* male were all male, no female sex;

then when this male hybrid was backcrossed with *H. armigera* female, the sex ratio of the backcross offspring was distorted as 1 ∶ 4. Haldane's rule stated that when in the F_1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous sex[13]. This rule applies in the case of hybridization between *H. armigera* female and *H. assulta* male, and therefore, it is proved that the sex chromosomes of *Helicoverpa* species are ZW type, and the female is the heterozygous sex. Absence of female hybrids in cross of *H. armgera* female and *H. assulta* male may result from sex chromosome-based meiotic drive. Hurst and Pomiankowski[14] suggested that Haldane's rule only occurred in taxa with sex chromosome-based meiotic drive, and chromosomally located genes may alter the sex ratio. Anyway, cytoplasmic factors can also alter normal sex ratios in insects[15]. Sterility of the male hybrids in the cross of *H. subflexa* female and *H. virescens* male clearly violates Haldane's rule because the male of *Heliothis* is actually homozygous sex. One possible mechanism of the hybrid sterility is based on deficiency of mitochondria derived from the mother *H. subflexa* in the bybrid and backcross[16]; another potential mechanism is based on incompatibility caused by interactions between maternally inherited microorganisms and the paternal genetic material in the nucleus[17].

No indication of hybrid sterility was detected by reciprocal hybridization between *H. armigera* and *H. assulta* followed by backcrossing of the hybrids to *H. armigera*. However, the only male hybrid of *H. armigera* female and *H. assulta* male was produced, and the sex ratio of the following backcross offspring was distorted; moreover, this hybrid also showed heterosis in the pupal weight and adult activity, therefore this hybrid has potential for genetic control of *H. armigera*, but further researches such as mass production of the hybrid and competition of the hybrids with the wild *H. armigera* male in searching and mating, need to be carried out before this hybrid could be released in the field for genetic control of *H. armigera*.

Acknowledgements We thank J. H. Zhang and L. H. Yin for assistance in the experiment, and L. X. Wang for rearing the insects. This work was supported by the National Natural Science Foundation of China (Grant No. 39670492) and the Chinese Academy of Sciences (Grant No. KSCX2-1-02).

References

1. Chen, Y. X., Fauna Sinica Vol. 16: Insecta, Lepidoptera, Noctuidae (in Chinese), Beijing: Science Press, 1999, 145.
2. Fit, G. P., The ecology of *Heliothis* species in relation to agroecosystems, Ann. Rev. Entomol., 1989, 34:17.
3. Guo, Y. Y., Progress in the researches on migration regularity of cotton bollworm and relationships between the pest and its host plants, Acta Entomologica Sinica, 1997, 40 (suppl.): 1.
4. Hoy, M. A., Insect Molecular Genetics: An Introduction to Principles and Applications, New York: Academic Press, 1994, 267.
5. Knipling, E. F., Use of insects for their own destruction, J. Econ. Entomol., 1960, 53: 415.
6. Laster, M. L., Interspecific hybridization of *Heliothis virescens* and *H. subflexa*, Environ. Entomol., 1972, 1: 682.
7. Proshold, F. I., Releae of backcross insects on St. Croix, U. S. Virgin islands, to suppress the tobacco budworm (Lepidoptera: Noctuidae): Infusion of sterility into a native population, J. Econ. Entomol., 1983, 76: 1353.
8. Laster, M. L., Sheng, C. F., Search for hybrid sterility for *Helicoverpa zea* in crosses between the north American *H. zea* and *H. armigera* (Lepidoptera:Noctuidae) from China, J. Econ. Entomol., 1995, 88(5): 1288.
9. Wang, N. C., Li, Z. H., Studies on the biology of cotton bollworm (*Heliothis armigera* Hübner) and tobacco budworm (*H. assulta* Quenee), Journal of Shandong Agricultural University (in Chinese), 1984(1-2): 1.
10. Wu, K. J., A lucerne-wheat germ diet for rearing the cotton bollworm, *Heliothis armigera* (Hübner), Acta Entomol. Sinica, 1985, 28: 22.
11. Hardwick, D. F., The corn earworm complex, Mem. Entomol. Soc. Can., 1965, 40: 9.
12. Laster, M. L., Hardee, D. D., Intermating compatibility between north American *Helicoverpa zea* and *Heliothis armigera* (Lepidoptera: Noctuidae) from Russia, J. Econ. Entomol., 1995, 88(1): 77.
13. Haldane, J. B. S., Sex ratio and unisexual sterility in hybrid animals, J. Genet., 1922, 12: 101.
14. Hurst, L. D., Pomiankowski, A., Causes of sex ratio bias may account for unisexual sterility in hybrids: A new explanation of Haldane's rule and related phenomena, Genetics, 1991, 128: 841.
15. Hurst, L. D., The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals, Biol. Rev., 1993, 68: 121.
16. Miller, S. G., Huettel, M. D., Davis, M. B. et al., Male sterility in *Heliothis virescens* × *H. subflexa* backcross hybrids, Evidence of abnormal mitochondrial transcripts in testes, Mol. Gen. Genet., 1986, 203: 451.
17. Krueger, C. M., Degrugiller, M. E., Narang, S. K., Size difference among 16S rRNA genes from endosymbiotic bacteria found in testes of *Heliothis virescens, H. subflexa* (Lepidoptera: Noctuidae), and backcross sterile male moths, Fla Entomol, 1993, 76: 382.

(Received June 29, 2000)