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Interpretation of the biological species concept from interspecific hybridization of two *Helicoverpa* species

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The biological species concept defines species in terms of interbreeding. Interbreeding between species is prevented by reproductive isolation mechanisms. Based on our results of interspecific hybridization between *Helicoverpa armigera* and *Helicoverpa assulta,* reproductive isolation mechanisms of the two species are analyzed. A combination of prezygotic factors (absent sex attraction and physical incompatibility of the genitalia) and postzygotic factors (female absence and partial sterility in F_1 hybrids) causes reproductive isolation of the two species. In addition, the role of interspecific hybridization in speciation is discussed.

biological species concept, reproductive isolation, interspecific hybridization, *Helicoverpa armigera, Helicoverpa assulta*

Species is a fundamental natural unit, but it has many different concepts: biological species concept, morphological species concept, evolutionary species concept, phylogenetic species concept, ecological species concept, and so on. Among them the biological species concept emphasizing reproductive isolation is most widely accepted, especially among zoologists[1]. Since the paper entitled "Interspecific hybridization of *Helicoverpa armigera* and *Helicoverpa assulta*" was published in *Chinese Science Bulletin*[2], my colleagues and I have been carrying out further comparative researches among the two species and their hybrids, including sex ratio and characteristics[3,4], sex pheromone biosynthesis in female[5], behavioral and physiological responses of male to sex pheromones[6], and larval feeding preference[7,8]. During this period of time, many readers and colleagues often raise and argue on the following questions: considering *H. armigera* and *H. assulta* can interbreed to produce viable offspring, why do not they belong to one species? Does this conflict with the biological species concept? The present article will try to deal with these questions according to what we know so far.

In 1969, Mayr[9] defined a species as follows: "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups". Obviously, the biological species concept defines species in terms of interbreeding. Here, the criterion of interbreeding refers to free gene exchange among populations in nature. Many interspecific hybridizations that can be induced between different species produce sterile offspring, or no offspring at all. However, there are also instances that different species are induced or forced to mate and yield partly or fully fertile offspring in laboratory conditions, but rarely or never hybridize in their natural environment. Interspecific hybridization of *H. armigera* and *H. assulta* is just an example of the latter.

Reproductive isolation among species has many mechanisms. Several main types of isolating mechanism include species living in different habitats, having a different courtship, no fusing of gametes, hybrids inviability or sterility. According to Dobzhansky's classification[10], reproductive isolation mechanisms are grouped into two major classes, prezygotic isolating

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Table 1 Reproductive isolation mechanisms of *Helicoverpa armigera* and *Helicoverpa assulta* according to Dobzhansky's classification[10]

Classification of reproductively isolating mechanisms	Isolating mechanisms of *H. armigera* and *H. assulta*
1. Prezygotic isolation mechanisms prevent the formation of hybrid zygotes	**Exist**
(a) Ecological or habitat isolation: the populations occur in different habitats in the same region.	No. The host plant range is significantly different, but they share some plants as host-plants, such as tobacco.
(b) Seasonal or temporal isolation: mating occurs at different times of day and night or at different seasons.	No. Calling period of females in the scotophase overlaps largely.
(c) Sexual or ethological isolation: mutual attraction between the sexes of different species is weak or absent.	Yes. Two components of sex pheromone are the same, but their ratios are just reverse.
(d) Mechanical isolation: physical non-correspondence of the genitalia prevents copulation.	Yes, but incomplete. The size and distribution of spines on aedeagus are different, and some mating pairs could not separate after copulation.
(e) Gametic isolation: the gametes or gametophytes of one species may be inviable in the sexual ducts of other species.	No. The spermatozoa are viable in the sexual ducts of another species.
2. Postzygotic isolation mechanisms reduce the viability or fertility of hybrid zygotes	**Exist**
(f) Hybrid inviability: hybrid zygotes have reduced viability or are inviable.	Maybe. There are no female F_1 hybrids when female *H. armigera* crosses with male *H. assulta*.
(g) Hybrid sterility: the F_1 hybrids of one sex or of both sexes fail to produce functional gametes.	Partial. There are about half of sterile F_1 hybrids when female *H. armigera* crosses with male *H. assulta*.
(h) Hybrid breakdown: the F_2 or backcross hybrids have reduced viability or fertility.	Partial. Some sterile individuals and skewed sex ratio occur in F_2 and backcross generations.

mechanisms and postzygotic isolating mechanisms (Table 1). To the best of our knowledge, isolation between *H. armigera* and *H. assulta* is not due to only one factor on Dobzhansky's list, it is caused by a combination of several prezygotic and postzygotic factors (Table 1).

The main mechanism of their prezygotic isolation is sexual or ethological isolation. The difference in ratio of sex pheromone components in female and behavioral responses of male to sex pheromones result in absence of mutual attraction between the sexes of the different species. *H. armigera* and *H. assulta* use (*Z*)-11-hexadecenal (*Z*11-16∶Ald) and (*Z*)-9-hexadecenal (*Z*9-16∶Ald) as their sex pheromone components but in reverse ratios, about 97∶3 and 7∶93 respectively. In *H. armigera*, *Z*11-16∶Ald is produced by Δ11 desaturation of palmitic acid, followed by reduction and terminal oxidation; *Z*9-16∶Ald results from Δ11 desaturation of stearic acid, followed by one cycle of chain shortening, reduction and terminal oxidation. Δ11 desaturase is the unique desaturase for the production of the two pheromone components. In *H. assulta*, palmitic acid is used as the substrate to form the major pheromone component, *Z*9-16∶Ald and the minor one, *Z*11-16∶Ald, and both Δ9 desaturase and Δ11 desaturase are involved in the biosynthetic process[5]. Results of flight tunnel tests showed that a large proportion of males of *H. assulta* exhibited a behavioral response to their own sex pheromone blend whereas they did not respond to the sex pheromone blend of *H. armigera*. Males of *H. armigera* exhibited behavioral responses to sex pheromone blends of the two species[6]. However, the further behavioral test proved the males of the two species only had attractive responses to conspecific calling females, no responses to heterospecific calling females (unpublished data).

Another prezygotic isolation mechanism is mechanical isolation. In the laboratory condition, the conspecific mating rate of *H. armigera* and *H. assulta* is 67% and 48% respectively. However, the mating rate of female *H. armigera* × male *H. assulta* and female *H. assulta* × male *H. armigera* is 10% and 12% respectively. Some mating pairs in heterospecific crosses could not separate after copulation, and this may result from the morphological differences in their genitalia. In the uneverted vesica of *H. armigera* the larger spines appear distributed equally along the aedeagus, but in *H. assulta* the larger spines appear in the posterior 2/3 of the aedeagus[11].

H. armigera and *H. assulta* are also maintained by postzygotic isolation mechanisms. In the laboratory, the cross between female of *H. armigera* × male *of H. assulta* yielded only fertile males and sterile individuals lacking a penis, valva or ostium bursae, but the cross between female of *H. assulta* × male of *H. armigera* yielded fertile offspring of both males and females[2−4]. This incomplete postzygotic isolation phenomenon follows Haldane's rule, which states 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[12]. 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 the incompatibility of cytoplasmic factors from *H. armigera* and the Z chromosome from *H. assulta*[3].

Therefore, *H. armigera* and *H. assulta* are reproductively isolated from each other, and certainly are different species. Production of fertile offspring in hybridization of the two species indicates that they are closely related species, but does not automatically mean that we must rename them as one single species. Rare examples of hybridization are not a challenge to the biological species concept, and on the contrary they prove the validity of the biological species concept.

In fact, at some region hybridization occurs when genetically distinct populations meet and mate, resulting in at least some offspring of mixed ancestry. The region is called a hybrid zone. The populations on either side of the hybrid zone may be different enough to have been classified as separate species. Such an area is more frequently found in plants than in animals. In some cases, hybridization may be the source of new adaptations or even of new species, so regions where hybrids are common are of more interest. Speciation is generally regarded to result from the splitting of a single lineage. However, at least in part of the hybrid zone, the hybrids themselves may evolve reproductive isolation from the parents, and become a third species. The butterfly species *Heliconius heurippa* is known to have an intermediate morphology and a hybrid genome. Mavárez et al.[12] have recreated its intermediate wing colour and pattern through laboratory crosses between *H. melpomene*, *H. cydno* and their F_1 hybrids. They then used mate preference experiments to show that the phenotype of *H. heurippa* reproductively isolates it from both parental species. There is strong assortative mating between all three species, the wing pattern and colour elements are both critical for mate recognition by males among them[13]. Such a case may not occur in our system because the sex chemical communication systems of the fertile F_1 hybrids were all similar to that of *H. armigera*[6].

Although the biological species concept emphasizes reproductive isolation, extremely rare hybridization and hybrid zones that restrict gene flow are compatible with the concept. Interspecific hybridization between *H. armigera* and *H. assulta* is a good example. However, the domain of the biological species concept is restricted to sexual, outcrossing populations and to short intervals of time, so it does not apply to asexually reproducing organisms or to populations widely separated in geological time[14]. For fossil species and asexually reproducing organisms we need to use other species concept (e.g. the morphological species concept).

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