The recent disciplinal progresses of agricultural entomology in China

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Abstract: In this paper, four recent advances and achievements of China in agricultural insect research, namely, on the genome of silkworm (*Bombyx mori* Linnaeus), on the geographical differentiation and regional migration of cotton bollworm (*Helicoverpa armigera* (Hübner)), on the standardized monitoring techniques for safety of honey bee (*Apis mellifera* Linnaeus) products, and on the virus transmission property of small brown planthopper (*Laodelphax striatellus* (Fallén)) as well as the interactions between vector and rice stripe virus (RSV), were reported. All of these researches are very important for controlling agricultural insect pests and the diseases they transmit, accelerating the molecular biological research of silkworm, and promoting the international trade of honey bee products. Most of these achievements mentioned above have got the national, provincial, ministerial or municipal awards on science and technology.

Key words: recent advances and achievements of China; agricultural insect research; control agricultural insect pests and diseases; molecular biology research; international trade

1 Introduction

Agricultural entomology is the most ancient and important secondary discipline of plant protection; it contains the research and technical extension on population dynamics, predictive methods and control strategy of agricultural insect pest (including harmful mite); development of economic insect production (such as silkworm (*Bombyx mori* Linnaeus) and honey bee (*Apis mellifera* Linnaeus) rearing and production of natural silk and bee products, etc.); and the use of predators or parasitoids against pest insects. Thus it can be seen that the progress of this discipline is of great significance to bio-safety and sustainable development of agriculture. In this paper, four recent advances and achievements of China in agricultural insect research dealing with the genomics of silkworm, geographical differentiation and regional migration of cotton bollworm (*Helicoverpa armigera* (Hübner)), standardized monitoring techniques for safety of honey bee products, and virus transmission property of small brown planthopper (*Laodelphax striatellus* (Fallén)) were reported as follows.

2 Advance in silkworm genome research

Silkworm is one of the important economic insects in China. As early as 1906, its 1st generation hybrid has already been used by Chinese silk growers to increase silk production, a typical example in agriculture to make use of heterosis. Despite the considerable advance in sericulture science and technology in recent tens of years, people still await new breakthrough in silkworm production. *B. mori* is the model of lepidopteran, and most of the farmland, forest and sanitary insect pests belong to Lepidoptera, so the advance in silkworm research is also significant for controlling lepidopterous pests. The silk gland of silkworm is an efficient protein factory and also a desirable bioreactor. Owing to its many-sided significance, the genome of silkworm has become an international hot spot of scientific research. In China, the studies on silkworm genome have been subsidized by a series of national projects such as the “863” Project, “973” Project, NSFC (National Natural Science Foundation of China), “948” Project, and National Sustentative Project, etc.

In 2001, a preparatory meeting of the international lepidopteran genome project (ILGP) was convened in Lyon by Japan, USA and France, attempting to promote the illustration of in toto genome sequence of important lepidopteran—silkworm. More than 20 scientists from 8 countries attended this meeting, but China had not been invited. In order to contend for international cooperation, China started a large-scale project of silkworm expressed sequence tags (EST). The results were reported at the 2002 ILGP meeting convened in Tsukuba by Japan, and the meeting defined that

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both China and Japan would be in charge of the international silkworm genome project. Nevertheless, no long after this meeting, the Japan side implied the meaning that they wanted to get the intellectual property right themselves, so they refused international cooperation in this field, and unilaterally started silkworm genomic sequencing since March 5^{th} 2003. Confronted with the rigorous competition, the Chinese scientists had no choice but to apply for the governmental support to launch the Chinese silkworm genome project. After they made great efforts and surmounted a lot of difficulties, the first genomic framework map of silkworm (also of lepidopteran) in the world was fulfilled and published on November 15^{th} 2003, which symbolized that the research on silkworm and Lepidoptera has stepped into a new phase.

2.1 Silkworm expressed sequence tags project

Silkworm EST project is the important part of SGP (silkworm genome project) and basic work of functional genome research. In 2002, the scientists of Southwest University started a large-scale silkworm EST project, by using the silkworm variety "Dazao" as test material, the cDNA libraries of main silkworm organs, including silk gland, midgut, fat body, hemocyte, seminal reservoir, ovary, virgin egg, diapause egg, non-diapause egg, and pupal fat body, etc. were constructed and 82 thousand EST sequences were obtained (so far the EST sequence number has been up to 200 thousand). It becomes the largest databank of silkworm EST in the world, and provides high-valuable resources and basic achievements for annotating silkworm genome and functional genome researches.

2.2 Framework map and fine map of silkworm genome

The in toto genome sequences of silkworm variety "Dazao" were tested with "shotgun" method by Southwest University and Beijing Institute of Genome Research, Chinese Academy of Sciences. They have fulfilled 5.5 million sequencing reactions, and finally, the in toto silkworm genome was covered with a coverage 5.9 times as big as the genomic size. The size of silkworm genome was 428.7 Mb, 2.6 times as big as that of pomace fly (*Drosphila melanogaster*) genome and one seventh of that of human genome. The forecasting gene number of silkworm was 18 510. A lot of important functional genes such as the peculiar gene of silk gland, genes related with metamorphosis and development, resistance and immunity, wing pattern formation, sexual determination and chromosome structure, etc. were discovered and identified. The number and size of silkworm genes were more and bigger than those of *D. melanogaster*, respectively. The contrastive results between genomes of the two insects showed that there were a large number of transposons inserting in the introns of silkworm gene which caused it even bigger than that of *D. melanogaster*. Based on the annotative analysis of silkworm movable factors, it was found that the increase of silkworm genome volume was an event happened recently, completely different from the former results generally found in genome analysis of human being and rodent. The contrastive results of functional domains of silkworm, fly and anopheline mosquito showed that among the 8 947 functional domains, 2 565 were common in these three insects, and 1 793 were peculiar in silkworm. Consistent with the transposon dilatation mentioned above, the functions of reverse transcriptase, integrase and transposase in silkworm genome were quite common. All of these important discoveries and the "treasure-house" reserved in genome itself will become the significant resources sustentation of basic science and industrial development. The framework map of silkworm genome is the biggest genome mapping accomplished by China alone, following the accomplishments of 1 % of human being genome and framework map of rice genome. The related article was published in *Science* [1].

The fine and completive genome maps are important scientific targets of genomics and also the bases of functional/applied researches. In 2006, the silkworm lab of Southwest University cooperated with Japanese scientists to uniformly piece together, assemble and annotate all the sequences and mapping information from different international organizations, and compose a high quality fine map of 9 in toto silkworm genome, its coverage of gene was up to 99.6 %. Later on, they have fulfilled the integration of fine map and molecular linkage map, and located 82.2 % of the total genes on the chromosomes, and also built up the ligation of silkworm genome and classical silkworm genetics. This achievement will be published in the near future.

2.3 Research on silkworm functional genome

Along with the accomplishment of silkworm genome framework mapping, its functional genome research has reached a rapid growing period. In consideration of the key problems occurring in basic theory and industrial development of silk production, the scientists of Southwest University identified and cloned a series of functional genes closely relative to economic properties such as synthesis of silk fibroin, resistance to diseases, sexual determination, development and metamorphosis, etc. Meanwhile, the gene regulation network was studied, the molecular mechanism of important economic property formation was illustrated, and the technical systems of silkworm transgene and molecular improvement were constructed. Furthermore, by applying transgenic techniques, the possibilities were explored for establishing biological factory with silk gland of silkworm as carrier, and developing biological pharmacy as well as entomological industry.

As a highly peculiarized organ of silkworm, the main function of silk gland is to produce silk fibroin. The highly efficient protein synthesis mechanism is not only a basic scientific problem, but also one of the most concerned topics of silk industry. The analytical results of in toto silkworm genome and gene expression map showed that there were total 1 874 peculiar genes of silk gland, in which a considerable part of genes such as those of ATP energy metabolism, synthesis and transportation of amino acids, gene transcription factors, protease and hormone regulator, etc. were relative to the secretion of silk fibroin. Moreover, they had found the evidence of hormone activity in silk gland. Real-time PCR (polymerase chain reaction) analysis of the transcribed product of silk fibroin structural gene showed that, the expression amount of silk fibroin gene in the rear part of silk gland of high silk output variety "Qiubai" was about 50 % more than that of low silk output variety "Dazao". In the rear part of silk gland of "Qiubai" variety, its transcription proportion of silk fibroin genes *fib-H*, *fib-L* and *P*25 was nearly 2∶2∶1, while in that of "Dazao" was about 5∶5∶1, and the two transcription proportions separately remained stable within different larval instars of the two varieties. It was found that, to maintain normal transcription proportion of silk fibroin gene is very important for synthesizing and secreting microbody of silk fibroin.

The immunity and microbe resistance are significant for silkworm breeding and pest control. After contrastive analysis of genome, 104 genes relative to immunity were obtained, including 35 antimicrobial peptides such as cecropin, 45 model recognition receptors such as peptidoglycan recognition protein, and more than ten signal transmission factors such as Toll, MyD88, Pelle, Tube and Dif, etc. from the signal transmission ways Toll and Imd[2-6]. 48 expression messages of immunity relative genes were got from the analysis of in toto genome chip expression map of the 5th instar silkworm larva. The main organs of insect possessing immune function are cuticle, fat body, midgut and hemocyte. The research showed that the large quantities of antimicrobial peptide gene and model recognition receptor expressed mainly in cuticle and fat body, and the signal transmission factors such as Toll receptor expressed not only in midgut, but also both in female and male gonads. No expression signals of these genes were detected in middle and rear parts of silk gland. The antimicrobial peptide genes of five families: Moricin, Nuecin, Cecropin, Gloverin and Enbocin, and immune system relative genes: *Bmspz* and *BmGNBP*3, were mainly separated and cloned. After injecting Gram-negative/Gram-positive bacteria and fungi into haemolymph to mutagenize silkworm, the important blood antimicrobial genes such as cecropin, antimicrobial peptides and immunoglobulin-like genes were studied at the mRNA level to reveal their expression after mutagenesis, and explore the importance of these genes for defense of silkworm against diseases, as well as to seek new way and theoretic sustenance by using transgenic or transgene-interference techniques for creating and breeding super-healthy silkworm varieties.

The sexual determination of silkworm is not only a basic problem of science, but also of great value at the industrial and pest insect control levels. Owing to that the most part of nourishment for female silkworm is used to produce eggs, so the breeding of male silkworm alone can reap much more economic benefit. By referring to the sexual determination way of *D. melanogaster*, 22 candidates of silkworm sexual determination genes were identified. It was found that in primary signal genes of sexual determination, *da*, *emc*, *gro*, *sisB*, *run* and *dpn* but not *her*, *sisA* and *sisC* had homologues in silkworm; while in dosage compensation way, the homologues of *msl*3, *mle* and *mof* but not those of *msl*1 and *msl*2 were found in silkworm; it was further indicated that silkworm lacks the dosage compensation mechanism. In body cell sexual determination way, all the genes: *Sxl*, *tra*2, *dsx*, *ix*, *Rbpl*, *doa*, *snf*, *vir* and *fl*(2)*d*, except *tra*, had homologues in silkworm, reflect that the body cell sexual determination in silkworm is probably rather conservative, only very rare genes and their regulation have changed. In the ways of copulation behavior and pedigree sexual differentiation, all the genes: *fru*, *dsf*, *ovo* and *out* possessed corresponding homologues in silkworm, which indicated that the two ways are probably very conservative. The results of gene chips detection for differentiating expression of male/female sexes showed that the expression of candidate genes: *gro*, *sisA*, *dsf* and *runt* raised in ovary, and those of *eme*, *Sxl*, *ix*, *fru* and *ovo* raised in seminal reservoir. These researches provide significant clues for illustrating the mechanism of silkworm sexual determination.

The large scale gene screening, investigation and identification techniques are very important for functional genome research. In accordance with silkworm genome framework map and EST sequences, an oligonucleotide microarray chip covering in toto silkworm genome was designed and made[7]. 22 987 probes were designed and arranged on aldehyde chip, each chip has 48 microarrays, and each microarray has 23 × 22 probes, it means one chip is big enough to arrange all the forecasting genes of silkworm. This chip was effectively used to study the silkworm gene expression map, and the chips serving other purposes were also developed.

By using the silkworm genome chip, the tissues, organs and time expression map of in toto silkworm genes were analyzed, and the gene expression characteristics at different development stages: embryogenesis, different larval instars, different developmental periods of pupa and adult, as well as important tissues and organs: midgut, fat body, hemocyte, seminal reservoir, ovary, silk gland, were obtained, and the peculiar expression genes of different stages and organs were further found or verified[8]. Based on the data of expression map, the databank of silkworm chips and gene expression BmMDB was established. The chip data and research results were already published in the world.

The microRNA is a very short uncoded RNA sequence, possessing regulative function for many biological processes. After analyzing the biological information of in toto silkworm genome, nearly 200 microRNAs were found and identified, and dealt mainly with the screening of 120 forecasting microRNA as probes. Meanwhile, the expression chip of silkworm microRNA was successfully made, its tentative expression map was obtained, and the functions of some given silkworm microRNA such as *let-7* were analyzed and found that they probably have functions for regulating the metamorphism and development of silkworm.

3 Differentiation of geographical types and regional migration regularity of cotton bollworm Helicoverpa armigera (Hübner)

Cotton bollworm is one of the important pest insects causing agricultural disaster, its facultative migration behavior is a major biological basis of regional catastrophe[9]. During its occurrence process, the long distant migration is effective for providing insect source, enlarging damage area and enhancing the level of disaster. To warn in time and accurately forecast the occurrence and damage are the cruxes of cotton bollworm control, while to clarify the regularity of migration is the key to the question for constructing its regional monitoring/warning systems. The catastrophic regularity of *H. armigera* had not yet ascertained in the past in China, which caused frequent cotton output reduction, brought about by forecasting mistakes and unpunctual control events. In view of the severe situation, systemic researches on diapause characteristics, genetic differentiation, cold hardiness/overwintering, niche adaptation, facultative migration and interregional migration behavior of cotton bollworm in different climatic regions were conducted from early 1990s up to now by scientists of Institute of Plant Protection, Chinese Academy of Agricultural Sciences and their collaborators, subsidized by "973" Project, Climbing Project, Surmounting Project, Contingency Project and NSFC, etc.

3.1 Differentiation of geographical types

3.1.1 Genetic differentiation of geographical populations

Numerical value analysis was conducted to determine the morphological variations of pupal and adult cotton bollworm and its sibling species, the results showed that there were significant variations among the individuals in a population; while only minor variations among different populations indicated that the geographical differentiation of cotton bollworm was negligible. Further, cross- and backcross-tests were carried out between cotton bollworm populations collected from Hengyang, Hunan and Hami, Xinjiang (two places have a long way away from each other), the results showed that the laying amount, hatching rate and laying time of egg of the two populations were similar, indicated that the genetic differentiation of *H. armigera* populations among different climatic regions has not yet come up to subspecies level. The results of allozyme analysis revealed that there exists very high genetic polymorphism in cotton bollworm population and without gene exchange obstruction among populations, all of them has made up a big colony capable of random copulation[10].

3.1.2 Differentiation of diapause characteristics in geographical populations

The research on diapause induction factors of cotton bollworm showed that the food quality had nearly no inductive action whilst the light rhythm and temperature were major decisive factors of its diapause; high temperature had obviously inhibited the occurrence of diapause; larval stage was sensitive to diapause signal; the diapause rate increased with the time of short day treatment. No significant influence of short-day treatment on the developmental velocity of larvae, the changes of water content in larva and pupa were negligible, and the heredity of diapause inductive characteristics was stable. Temperature was the major factor for its diapause relieving. The induction and relief of diapause in cotton bollworm were two relatively independent processes; the condition of diapause induction had no relation to that of diapause relief. The diapause pupa can be relieved under high temperature, but the time of need was rather long. Low temperature can activate and relieve diapause pupa, and the duration of pupal stage was significantly shorter than that of high temperature treatment. In a population, the time of need for high temperature relief and low temperature activation among individual diapause pupae were rather heterogeneous, the activation of low temperature on diapause pupa caused the emergence pattern of adult cotton bollworm changed gradually from scattered type to aggregative type. The heredity of diapause duration was stable.

The light/temperature reactions for diapause induction of cotton bollworm populations were tested, the results showed that there were significant variations among the populations from different cotton regions. Under 20 ~ 26 ℃ temperature and 11 ~ 14 h, light period conditions, no diapause pupae occurred in Hainan population, which belongs to South China cotton region. Whilst the diapause reactions of all the populations from north part of middle and low reaches of Changjiang River, Yellow River Valley, most early ripeness cotton region in northeast China, and inland cotton region in northwest China, were very sensitive to light/temperature induction.

The diapause relief characteristics of geographical populations of cotton bollworm in different cotton regions were obviously distinct from each other. Along with the ascendant latitude, the development duration of diapause pupae under high temperature were significantly elongate, and the emergence pattern inclined to scattered. Low temperature treatment can obviously shorten the diapause duration of populations from north of Changjiang River Valley, but no evident activation on the populations from north part of South China Cotton Region was found, which indicated that there was also differentiation within diapause relief mechanism of cotton bollworm in China. The induction and relief mechanisms of diapause are important for existence of geographical cotton bollworm populations. On one hand, they need to avoid too early relief of diapause in autumn, on the other hand, they want to develop in time as the spring comes.

The study on diapause characteristic of Xinxiang population (in Henan Province) showed that there was rather strong heterogeneity among individuals, its population heredity maintained in a heterozygous state, the facts provided the indirect evidence that there existed gene interchange among different geographical populations. The heredity heterogeneity of diapause within population will directly affect on population dynamics of cotton bollworm, and it is probably the key factor of the appearance of the 5th generation cotton bollworm in several years in northern China.

Diapause is a major index for ecological adaptability of insect. All the critical light rhythm and duration of diapause, effective accumulated temperature needed for diapause relieving and the cold hardiness displayed in diapause process were determined by heredity. The diapause heredity of cotton bollworm was nearly half-recessive pattern, after cross between populations of North China and Changjiang River Valley, the diapause characteristic of their offspring was tending toward the latter population. Once the migration occurs, the gene interchange between the two regions will evolve, then the seasonal dynamics of cotton bollworm populations will be regulated by diapause characteristic; as the diapause rate of combined offspring decreased, the incomplete 5th generation will appear in North China Region, causing a large number of adults of cotton bollworm occur in late September and October, the season lack of host plant.

3.1.3 The cold hardiness differentiation of geographical populations

The undercooling points of diapause pupae of cotton bollworm from different ecological regions in China were tested, the results showed that there were significant differences among them. Overall, the undercooling points decreased with the increase of elevation and latitude, the differences of survival abilities under low temperature among these populations were distinct, the order of low temperature survival abilities of geographical populations was Xinjiang > North and Northeast China > Changjiang River Valley. Genetic analysis showed that the cold hardiness of diapause pupa was controlled by several imperfect dominant genes. This genetic pattern is capable to increase overwintering offspring number owing to the gene interchange between populations possessing strong and weak cold hardiness ability.

Investigations of overwintering behavior of cotton bollworm in North and Northeast China were conducted, and the results showed that cotton bollworm could not overwinter in the vast areas north of west and middle Liaoning Province, while it could successfully overwinter in sheltered ground and field of south parts of this province. Combined with the analysis of climatic characteristic, the north overwintering boundary of cotton bollworm in East China could be deduced as a coincidence of −15 ℃ isotherm of the mean lowest temperature in January. The trend of this boundary line from east to west was north of Dalian (Liaoning Province)→Qinhuangdao (Hebei Province) →Zhangjiakou

(Hebei Province) → Taiyuan (Shanxi Province) →… → Wuwei (Gansu Province). The north overwintering boundary of cotton bollworm in Changjiang River Valley will be south of the southern part of North China. No diapause occurred in cotton bollworm population in middle and south parts of South China cotton region, their overwintering region will be south of middle subtropics. In Xinjiang Uygur Autonomous Region, most of the farmland soil is yermosol with low soil moisture, thermal capacity and thermal conductivity. The January surface temperature is lower than air temperature in north part and somewhat higher in south part. In Kashgar and Hotan of south Xinjiang and Turpan Basin of east Xinjiang, the January mean lowest temperature is generally lower than −14 ℃ isotherm, and there will be the suitable regions for overwintering of cotton bollworm. According to the cold hardiness ability of cotton bollworm in Xinjiang, the January mean lowest temperature of the region including Aksu, Kuqa, Hami and partial south Xinjiang located between −16 ℃ and −14 ℃ isotherms, their daily extreme temperature in winter was about −20 ℃, a certain amount of cotton bollworm should be capable of overwintering there, so called risky overwintering region. The January mean lowest temperature and general January lowest temperature in other places in Xinjiang is lower than −20 ℃ and ranges from −25 ℃ to −35 ℃, respectively, far inferior to the super cooling point of diapause pupa of cotton bollworm, so it is difficult to overwinter there.

3.1.4 Adaptability of geographical populations to rainfall and temperature

The study on the effect of soil moisture content on emergence of pupal cotton bollworm showed that the pupae have 3 kinds of expressions in eclosion process: normal emergence, deformed emergence and died before emergence. Under 0 % ~ 80 % relative content moisture conditions, there were no significant differences among the adaptabilities of Henan, Zhejiang, Sichuan, and Hunan populations. Their cold hardiness abilities decreased obviously with the increase of soil moisture content, which indicated that soil moisture content is an important factor affecting on its overwintering. The relations between temperature and developmental rate among different populations were compared in laboratory, and the development dynamics of populations from Changjiang River Valley and North China under natural condition of Beijing area were simulated, the results showed that as the temperature ranged from 20 ℃ to 30 ℃, there were no differentiation response to temperature among development rates of different geographical populations, while the distinction between dynamics of diapause pupa from the two regions was notable. The results of integrated analysis indicated that Beijing area should be the north boundary of north China ecological region of cotton bollworm, the cotton bollworm from Changjiang River Valley could not adapt to the yearly change of light rhythm and temperature, so it was incapable to fulfill its yearly life cycle under climatic condition of Beijing.

Based on the researches mentioned above, cotton bollworm in China was divided into tropical, subtropical, temperate and Xinjiang types, which had highly specialized adaptability to south China, Changjiang River Valley, Yellow River Valley and Xinjiang regions, respectively. The tropical type cotton bollworm mainly distributed in the region south of 22° N, the subtropical type in 22° N ~ 32° N of Changjiang River Valley, the temperate type in that from north of 32° N to south of −15 ℃ isotherm of mean daily lowest temperature in January, and the suitable ecological region of Xinjiang type located in south temperate area of middle and south parts of Xinjiang Uygur Autonomous Region.

3.2 Facultative migration biology and migratory route

3.2.1 Flight biology of cotton bollworm

By using insect flight mill, the flight ability of cotton bollworm was tested, the results showed that the flight ability of 3 ~ 6 days old adult was the strongest, the mean accumulative total flight time and mean flight distance of continuous suspending flight of 6 days old moths were 41.3 h and 239.7 km, respectively. The moth selected flight with the wind in annular wind tunnel, as the wind speed enhance, it gradually reduced the flight velocity itself and depended on the carrying forward of airflow. The detection under electro-microscope showed that similar to oriental armyworm *Pseudaletia separata* Walker and black cutworm *Agrotis ypsilon* Rottemberg, the flying muscle structure of cotton bollworm was suitable for long distance flight; the main energy reserve for flying was glyceride-like materials. During flying, the content of protein changed negligibly, while the content of glyceride and glycogen was negatively related to flying time. During early flying stage, the content of glycogen decreased quickly, then the decrease of glyceride content gradually enhanced. The 38.87 h flight had consumed energy of 218.72 J, in which the proportions of glyceride and glycogen were 85.87 % and 14.13 %, respectively[11].

3.2.2 Radar observation of migration

It was found by radar observation that cotton bollworm began takeoff at dusk (half an hour after sunset), and the largest density area occurred at about half an hour after takeoff[12]. The density of cotton bollworm in the sky decreased quickly along with the mass emigration of local population; as there passed through a large amount of migratory population, the density of cotton bollworm revealed fluctuant change. Ordinarily the migratory cotton bollworm swarm alighted at dawn (half an hour before sunrise). The continuous flying times changed with different seasons, they were about 9.5, 7, 8.5 and 10 h in spring, late spring to early summer, summer and autumn, respectively. Precipitation was the main factor causing takeoff and alighting of cotton bollworm, the rain at dusk impeded takeoff of local population (including local emerged and immigrated one day before). If the rain came down after its takeoff and migrated with wind in high altitude (in midnight), the heavy rain forced migratory swarm of cotton bollworm gathered and alighted in raining area, but only little effect of light rain on its population migration. The migratory height of cotton bollworm was lower than 1 500 m, and its high-density migratory layer was about 300 m, the most warm air and most high speed airflow existed in this layer. Sometimes the rather weak migratory layer occurred at the heights about 500 m and 1 000 m, and formed multi-layer migration. During autumn back migration, the main migratory layer appeared more often at the height of 500 ~ 1 500 m, with the aid of current north wind to migrate towards south. In different seasons, cotton bollworm used favorable wind direction in different heights to realize long distance migration with the wind, the angle between wind direction and location displacement was less than 12°. Under strong wind power and stable wind direction conditions, cotton bollworm appeared obviously similar directional characteristic, i. e. appeared typical dumbbell-like echo on the screen; as under weak wind power and varying wind directions conditions, no dumbbell-like echo appeared on the screen.

3.2.3 Evidences of long distance migration

Resistance to pesticide is one of the genetic characteristics remained for insect by agricultural production and management of human. The increase of resistance of cotton bollworm to insecticide is the inevitable result caused by excessive use of chemical insecticide, and the resistance level of geographical population positively correlates with the use amount of insecticide. Therefore, to analyze the correlation between resistance level of geographical population and local use amount of insecticide is capable for inferring the migratory route of cotton bollworm. The resistance of more than 50 geographical populations from 5 major cotton regions to lamdacyhalothrin, endosulfan and phoxim were tested, the results showed that, generally, all the resistance of cotton bollworm from Changjiang River Valley Region, Yellow River Valley Region and Liaohe River Valley Region had already risen to a rather high level, while that of Northwest Inland Region still maintain at sensitive level, indicated that no obvious gene interchange appeared so far between the populations from Xinjiang Region and interior provinces, and also periphery countries such as India and Pakistan (according to external reports, the resistance of cotton bollworm in the two countries was quite high). In North China, cotton is mainly grown in its middle and south parts, where large amount of insecticides are used; but the major crops in its north and northeast parts are maize, rice and fruit trees, and the growing area of cotton is rather small, so the use amount of insecticide is far lower than that in the former parts. Nevertheless, the insecticide resistance of Liaoyang population from maize growing area of Northeast China and Beijing Suburb (no cotton growing) population was rather high and clearly unconformable to the local lower insecticide application. Based on the facts mentioned above, it can be inferred that the cotton bollworm population occurs in middle and north parts of Bohai Bay area should be from its south part by immigration. In order to confirm this inference, the migratory dynamics of its 1st generation over Bohai Sea was investigated on a passenger ship in 1996 – 1997, and found a large amount of cotton bollworm moth in the sky 20 ~ 80 km apart from coastline was migrating toward the direction to Liaoning Province. Commonly, the nocturnal insects alighting on passenger ship crawl out from covered positions at about 19:30, and then flap wings, take off and migrate to northeast direction (the direction of monsoon in summer). The time for takeoff process was about 30 min, and finished until 20:00. The real migratory time of cotton bollworm moth in the sky was about 8.5 ~ 9.5 h.

The North China Region located at south part of Bohai Gulf and the Northeast Region located at its north part, they separately belong to different climate zones, the distinct accumulated temperature between the two regions caused great phenological disparity. As a result, winter wheat and spring wheat, the main host plant of the 1st generation larval cotton bollworm, are respectively cultivated in the two regions, and the developmental progress of wheat in the latter region is significantly later than that in the former region[13]. Systematic investigation showed that the 1st generation population dynamics in Chaoyang and Liaoyang Cities of Liaoning Province (both located at north part of Bohai Gulf Region) were inconsistent with local phenological conditions while rather consistent with that in Langfang City of Hebei Province and west suburb of Beijing City. All of these facts indicated that the 1st generation adult cotton bollworm in Northeast Region emigrate from North China Region.

Adult cotton bollworm needs to feed on nectar plants for acquiring extra-nutrition, so the plant pollen becomes a natural labeling on cotton bollworm. To analyze the different blooming periods of plants and morphological peculiarities of pollens are capable of illustrating the migratory route of cotton bollworm. The pollens carrying on adult cotton bollworm, caught from North China Region and sea surface, were detected under electro-microscope, the results showed that 94.4 % of the pollens were carried on its proboscis, and a few on its compound eye; there were many variations in kinds and quantities of pollen carried on cotton bollworm collected at different areas and times, most of the pollens stemmed from Chinese jujube, Tamarisk, carrot, green thistle, feather cockscomb, sunflower, and several species of Composite Family, etc. It was found that the cotton bollworm moth collected from Bohai Sea surface in early-middle June had carried Chinese jujube pollen, in this time, this plant in Liaoning Province has not yet in-bloom, and that in middle and south China, its blooming period has already finished, indicated that the cotton bollworm moth caught from Bohai sea surface and migrated toward Liaoning ought to come from Yellow River Valley.

In 1996 - 2000, the relation between development progress of ovary and migration behavior of cotton bollworm was studied successively in Langfang City of Hebei Province, Liaoyang City of Liaoning Province and west suburb of Beijing City, the results showed that both the 1st generation populations from Langfang City located at south part of Bohai Gulf and Liaoyang City at its north part mainly immigrated from external, the 2nd and 3rd generation populations in majority of years were locally, but also external populations immigrated in a few years. All the 1st ~ 3rd generation populations trapping from the sky of west suburb of Beijing possessed physiological characteristic of migratory insect, indicated that all the generations have migratory behavior.

By applying external dyeing method, the 2nd and 3rd generation adult cotton bollworm were labeled and released in Xinye County, Henan Province, 100 recapturing points were randomly set up within a radius of 30 km surrounding releasing point as center, 16 labeling moths were recaptured, in which 61.54 % of them were caught in 1 km range, and 38.46 % were in 10 ~ 30 km range.

3.2.4 Mechanism of facultative migration

The flight abilities of cotton bollworm collected from cotton, maize, peanut, mung bean and sesame fields and those fed on artificial diet and different cotton organs in larval stage were tested with flight mill, the orderly range of different host plants was cotton and maize > peanut > mung bean and sesame, it basically reflected the order of nutritional value of host plants for larval cotton bollworm; and the flight abilities of individuals having better nutritional conditions such as artificial diet and cotton boll were several times as strong as those fed on cotton leaf. The more body weight of adult weighed out, the more strong flight ability it possessed, and the flight distance of moth acquiring extra-nutriting was twice as far as that no extra-nutrition obtained. The research also found that as the moth was short of extra-nutrition, the developmental progress of its ovary was significantly prolonged. Nevertheless, the experiments had proved that there was no relation between adult flight ability and larval density or nutritional condition in larval stage. In view of this, the long distant moving of cotton bollworm should be an important behavioral reaction of adult against harmful environment after emergence. Under high adult population density and lack of nectar plants or oviparous hosts conditions, cotton bollworm moth will adopt long distance migration strategy along with the air current.

3.3 Seasonal migration regularity

Cotton bollworm possesses tail wind directional behavior, and its migratory direction is closely relative to wind direction. The most wind direction during eclosion of the 1st generation adult in North China inclines to the north, so the moths are "transported" toward north. The wind speed and direction are changeable in different years, as in the year the continuous, stable and powerful southwest wind is current (such as in 2001, the highest wind speed was 20 m/s), cotton bollworm can migrate 150 ~ 300 km every night from North China Region to Northeast Region. Whilst in the year the wind speed is weak or the southwest and southeast wind occurs alternately, only partial adult can migrate from north part of North China Region to Northeast Region, and the great part of adults migrate within vast area of North China Region from south to north (northeast or northwest)[14].

The behavioral parameters of takeoff, alighting and sky movement of cotton bollworm were drawn into aerologic locus analysis, a forecasting model for suitably simulating the migratory locus and route of cotton bollworm was established. Radar observating and simulated model analysis showed that the 1st generation adult cotton bollworm in North China Region was capable of migrating from its north part into Northeast Region or internal movement in original region, the 2nd generation adult could migrate along with airflow into Northeast Region, and the 3rd generation adult could migrate from Northeast Region return to North China Region or from north part of North China Region return to its south part[15]. During eclosion of the 2nd generation cotton bollworm in North China Region, the tropical complex zone jumped between North China Region and Northeast Region, the wind direction and wind speed were changeable, as the wind direction was rather stable in this period, most of the 2nd generation adult immigrated into Northeast Region (its migration distance depends on wind speed), as the wind direction was changeable, only a small part of the 2nd generation adult migrated with southwest wind in the night into Northeast Region, and most of the adults detained and reproduced in North China Region. In autumn, in pace with the tropical complex zone pulled out southward, the partial west wind became current, which is unfavorable to the return migration of adult; but it is able to select the transient opportunity of north wind appearance or climb to the north wind blowing stratum for south migration[16]. The time span of migration in autumn was rather long, from middle August to early October, the appearance of migration peak was coincided with partial north wind.

4 Recent advances in standardized detection techniques for bee products in China

In modern international competition, the technical standard has already become a key guarantee and long-term strategic task for national benefit and safety. In China, the institution of technical standard for bee product safety is still in an initial stage. Before 2002, only one national standard for inspection of bee product safety and sanitation was set up. Henceforth, under the subsidization of National Bureau of Quality Inspection, many-sided explorations and great efforts were put in on the research and development of detection techniques and qualitative standards of bee products, and periodical advances were obtained, in which 33 national standards dealt with the techniques for identifying false and true honey and detecting residues of pesticides and veterinary drugs in bee products as well as the evaluation technique for their qualities, instituting by Qinhuangdao Bureau of Export/Import Inspection and Quarantine, were promulgated and implemented, and the safe production system of bee products, detective and inspecting measures and quality control system were preliminarily established[17-19]. All of these are of great significance to enhance the quality of bee products in our country and get rid of the technical barriers from developed countries.

A few years ago, the main problems on honey quality in China were excess residue caused by illegally use of drugs during beekeeping and confounding of immature honey or spurious "honey" during honey production. In pace with the more and more strict demand on safety and sanitation of honey products in international market, the bee products of our country exporting to Europe, America and Japan, the major honey markets in the world, have encountered endless technical ramparts, mainly dealt with the techniques for distinguishing the true from the false and detecting of foodstuff safety and sanitation. The discrimination of true/false honey needs isotopic mass spectrometric technique, at that time in China, it was still a technical gap in this field; whereas the inspection of food safety and sanitation concerned with nearly 700 chemicals, belong to 9 kinds of drugs: chloromycetin, sulphanilamides, tetracyclines, glucosaminides, macrolides, β-lactams, nitro-imidazoles, nitro-furans and quinolones, 5 kinds of pesticides: acaricides, organochlorines, organophosphorus, carbamates and pyrethrins as well as 3 kinds of micro-chemicals. Their physical/chemical properties, existent forms and residual amounts in bee product substrates varied immensely from each other, and the necessary precisions of quantitative detections attained to micro-(gram $\times 10^{-6}$) and even 10 × nano-(gram $\times 10^{-8}$) levels. There were 3 difficult problems need to settle in exactly quantitative detection of bee products, the first: what kind of extraction system can be adopted to completely collect the trace chemical residues and effectively separate them from bee product? the second: what kinds of separative and enriched techniques will be used to enrich target chemicals and reject large amount of interferential substances? the third: what detection technique can be adopted to accurately carry out quantitative analysis, and pledge to attain the precision of detection required by developed countries. Consequently, to analyze and prove the qualification of bee products conform to the requirement of international market is a strenuous and time-consuming task possessing high technical difficulty.

4.1 Distribution regularity of carbon isotope mass ratio of bee products in China

So far has the research on discrimination of foodstuff adulteration become a topical subject of food science. The USA government had published the first "Law of Pure Foodstuff" in 1906. The general techniques at present for studying truthfulness of honey are gas chromatography (GC), thin-layer chromatography (TLC), spectro-photometry, high performance liquid chromatography (HPLC) and isotope mass spectrography, in which, the latter is most praised in the world. After studied for 20 years (since 1970 - 1991), the USA government has established a standardized AOAC (Association of offical Analytical Communities) method.

The technical principle of carbon isotope ratio method for distinguishing true/false honey is in line with the fact that most of nectar plants make use of carbon-3 molecular cycle (also called Calvin Cycle) to conduct photosynthesis and finally produce carbohydrates (sugary materials), and these plants are known as carbon-3 plant. The carbon isotope ratios ($\delta^{13}C$ values) of bee honey range from -28 ‰ to -22 ‰. In nature, the carbon isotope ratio values of nearly all the nectar plants are in this range; whereas the plants producing high fructose starch syrup or cane sugar as well as certain other plants using carbon-4 molecular cycle (also called Hatch-Slack Cycle) to conduct photosynthesis, are known as carbon-4 plant, such as corn and sugarcane, the carbohydrates they produced are called as carbon-4 plant sugar, their carbon isotope ratios ($\delta^{13}C$ values) range from -20 ‰ to -10 ‰. The chemical structures of carbohydrates from carbon-3 and carbon-4 plants are similar, while their carbon isotope ratios are different. As the two kinds of carbohydrates are mixed together, the carbon isotope ratio will change with the proportion in mixture. The $\delta^{13}C$ value of pure honey is similar to that of its protein, after carbon-4 plant sugar is mixed into pure honey, the $\delta^{13}C$ value of mixture is changed, but the $\delta^{13}C$ value of protein is constant, because no protein contains in carbon-4 plant sugar, so we can use the $\delta^{13}C$ value of honey protein as internal tagging to distinguish whether or not the honey has been mixed with carbon-4 plant sugar, and calculate the amount it mixed.

In order to prove whether or not the theory mentioned above is an universal truth, from 1998 to 2001, the Qinhuangdao Bureau of Export/Import Inspection and Quarantine used stable carbon isotope ratio method to test the $\delta^{13}C$ values of 27 species of Chinese pure and single flower honey, 300 general survey samples of 7 species of major single flower honey and 12 990 commodity honey samples collected from more than 130 honey enterprises in 25 provinces of China, the results showed that the $\delta^{13}C$ values of Chinese honey ranged from -21.5 ‰ to -28.0 ‰. A conclusion was reached that the honey $\delta^{13}C$ value has basically no relation to natural environmental conditions (such as the difference of region, water and soil, weather, etc.) for nectar plant growing, but only slightly related to the species of nectar plants. The carbon isotope ratio values of most of the honey from different producing areas or different kinds of honey in China come within the range of theoretical values proposed by USA, indicated that the theory of honey carbon isotope ratio is suitable for Chinese honey. This study has provided the theoretical foundation for scientific identification of true and false honey, the national standard: GB/T 19832.1-2002 was established, and "Carbon Isotope Ratio Analysis, a Method for Determining Content of Carbon-4 Plant Sugar" was promulgated on Dec. 30th, 2002 and implemented on June 1st, 2003[20].

A long-term cooperation on the contrastive experiments of honey samples was conducted among the Technical Center of Qinhuangdao Bureau of Export/Import Inspection and Quarantine (China) and Europa Company of Science and Technology (UK), Honey Data Company (USA) and Bremen Institute of Honey Research (Germany). By means of the accuracy and impartiality of analysis results, the internal and external reputation of this technical center has been continuously enhanced. From 1998 to 2006, totaled 21 000 batches of honey samples delivered from nearly 400 enterprises in 28 provinces/cities were detected by this center. As an identification method for truthfulness of bee honey, the standard has already been placed in the compellent national honey standard: "Bee Honey GB 18796-2005".

4.2 On the residual detection techniques of 65 drugs in bee products

According to the requirement of external technical ramparts, a lot of experiments were carried out by using modern extraction, separation and enrichment techniques such as solid-phase microextraction, ion exchange, liquid-liquid partition chromato- graphy, gel osmosis, column chromatography, derived biochemistry, enzymatic chemistry and microwave extraction/decomposition, etc. to pretreat the samples and rationally compose new technique systems for sample preparations, explore and determine effective methods for extracting target chemicals and get rid of interferential materials from honey products. Further, in the light of the properties of different kinds of target chemicals, the suitable analytic techniques such as HPLC, GC, TLC, organic mass spectrograph, inorganic mass spectrograph, spectrophotograph, atomic absorption, emission spectrograph, ELISA (enzyme linked immunosorbent assay) and microbial identification were separately put together into different quantitative analysis methods for detecting the residues of 65 target chemical (including 61 antibiotics, phenol (wasp repellence), benzaldehyde, bromopropylate and 4,4-dibromobenzophenone (miticides)) belong to 9 kinds of drugs[21].

In accordance with the conditions to establish technical standard of analytic methods stipulated by the International Standardization Organization (ISO), International Union of Principled and Applied Chemists (IUPAC) and AOAC, the contrastive sample tests were conducted between this technical center and authoritative labs of USA, UK, Germany and Japan. The main countries import Chinese bee products, the quality of Chinese bee products had earned the international approval. 21 items of national standards for detecting the residues of drugs were instituted, the facts indicated that our analytic techniques for detecting drug residues in bee products has attained the world's most advanced level.

4.3 On the residual detection techniques of 614 pesticides in bee products

In order to further elaborate the latent capacity and enhance the sensitivity of HPLC-MS detection, increase the number of test pesticides, and ensure more rapid and exact examination, for satisfying the more and more strict demands of developed countries, in 2006, the standard GB/T 19426-2003 was divided into two independent standards: GB/T 19426-2006 and GB/T 20771-2006, separately suitable for GC-MS and LC-MS-MS analyses[22,23]. One time of samples prepared, the detectable pesticide number of GC-MS increased from 282 to 497, and that of LC-MS-MS increased from 22 to 420, while the detectable number of both two methods was 299, and the total amounted to 614. For the most part of pesticides, the sensitiveness of LC-MS-MS was higher than that of GC-MS, so the former method was more desirable for multi-residue analye of pesticides. So far, two data banks including 629 and 765 pesticides separately used for LC-MS-MS and GC-MS analyses were constructed.

Two national standards for multi-residual detection of pesticides in honey, 21 national standards for detecting residues of drugs in honey and 10 national standards of detection methods for carbon-4 plant sugar, high fructose starch syrup, fructose, glucose, canesugar, a number of metal elements, glycerol, hydroxymethylfurfural in honey, conductivity of honey and flavonoids in propolis, were laid down by Qinhuangdao Bureau of Export/Import Inspection and Quarantine. These national standards had initially made up the Chinese technical standard system of bee product quality, and evaluated as "come up to advanced world standards". In 2002 – 2006, the detected amount of bee products by this bureau was up to 200 kt, 52 % of the totaled bee products in China, which indicated that this system has enjoyed the trust of customers from home and abroad.

5 On the interaction between transmission property of small brown planthopper (Laodelphax striatellus Fallén) and rice stripe virus

Small brown planthopper (SBPH) is the vector of rice stripe (pathogen: rice stripe virus, RSV), rice black streak dwarf and maize rough dwarf (pathogen: rice black streak dwarf virus, RBSDV) and wheat rosette stunt (pathogen: northern cereal mosaic virus, NCMV). Since 1990s, maize rough dwarf disease prevailed in several provinces in Northeast, North China and Eastern China. Since late 1990s, rice stripe rampantly broke out in Eastern China, centered in Jiangsu Province. In 2004, 76 % of the rice fields in Jiangsu Province were infested by RSV, 50 % were seriously damaged, and 0.33 % was total crop failure[24,25]. For this reason, the occurrence and RSV transmission behavior of SBPH has aroused wide-spread concern and got subsidizations from national "863" Project, NSFC, National High Grain Output Engineering, National Sustentative Project and government of Jiangsu Province. In recent years, the research on interaction between SBPH and RSV carried out by the Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, has made considerable headway.

5.1 On RSV transmission property of SBPH

SBPH is capable of persistently transmitting plant virus such as RSV, RBSDV and NCMV, and it can also transmit RSV by viruliferous egg to its progeny. It can transmit RSV to rice plant only after 3 min of inoculation, the shortest inoculation time, and the general transmission time ranged from 10 to 30 min (Fig. 1)[26,27]. The 3rd to 5th instar nymphs possess high RSV transmissibility, which has somewhat decreased as they become adults, only very few male adult can transmit RSV. The transovarial RSV transmissibility is very high. Female adults possessing rather strong transmissibility are simultaneously prone to have higher transovarial transmissibility. For example, as the transmission rates of female adults were from 80 % to 100 %, 20 % and 0 %, the mean viruliferous rates of their progenies were 86.3 %, 40.6 % and 10.9 %, respectively. If no viruliferous individual was found in the progenies, it means no infecting potential exists in female adult and its progenies, namely, they have perfectly lost the RSV. The virus free SBPH can acquire RSV after fed on viruliferous rice plant, the indoor acquisition rate of RSV can attain to the same level as in rice field. The concentrations of RSV in SBPH varied among individuals, showing the variation in transmissibility and transovarial transmissibility of the vector. Generally, except the selected individuals having high transovarial transmissibility, the concentration of RSV decreased with the increase of generational order of SBPH. Commonly, after acquiring RSV from diseased plant, the transovarial transmissibility of SBPH is rather high. The fact of transovarial transmission not only provides powerful evidence: plant virus propagates in insect body, but also a real instance: virus vertically disseminates in insect body.

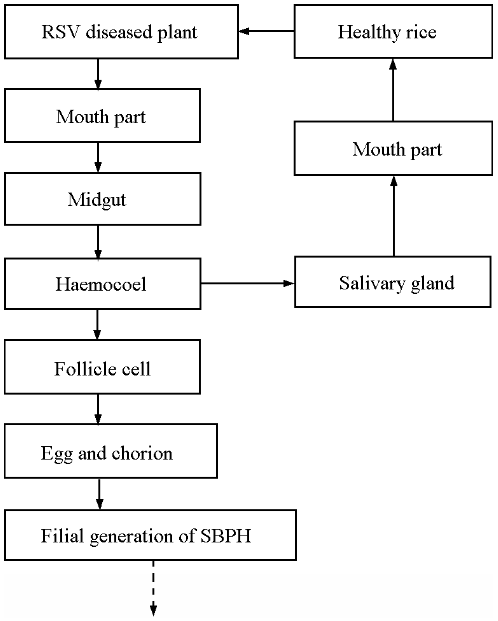

Fig. 1 Suggested way of transovarial transmission of RSV in SBPH

5.2 On the viruliferous immunoassay and interaction between SBPH and RSV

A viruliferous immunoassay kit for detecting SBPH was created by Jiangsu Academy of Agricultural Sciences; it is of great significance not only for accurately forecasting and controlling of RS disease, but also for studying the interaction between vector and virus. By applying Dot-ELISA method, the viruliferous situations of SBPH from different regions, diseased rice fields, generations, instars, families and their progenies were analyzed[28,29]. It was found that there were obvious differences among the viruliferous rates of SBPH populations from different regions, that from light diseased region was evidently lower, but no inevitable relation was found between the incidence of RS disease in sampling rice field and the viruliferous ratio of SBPH; which is probably related to the viruliferous situation of initially invasive SBPH population. The viruliferous ratios of overwintering, the 1st and the 2nd generations showed a "V" type development, implied that the viruferousness of SBPH in rice field had naturally weakened or accumulated effects. The viruliferous concentration in SBPH increased with the order of instar, and the lower concentration in neonatal nymphs is probably the main reason for the problem why their survival is similar to those of avirulent SBPH.

Tracing observations on the viruliferous ratios of different SBPH families and further on those of repurified progenies in partial families were carried out, and the results showed that the interaction between SBPH and RSV can be divided into 4 kinds: HA, high affinity, SBPH possesses stable transovarial RSV transmission ability; MA, middle affinity, it possesses stable transovarial RSV transmission ability only in a certain period; LA, low affinity, it can acquire RSV, but no stable transovarial RSV transmission ability; and NA, no affinity, it cannot acquire and carry RSV. The interaction between SBPH and RSV has been distinctly classified for the first time by the authors. It can partially explain the prevalent property of RSV: outbreak, intermittence and migration.

5.3 Location of RSV in SBPH

By using transmission electron microscope and immunogold technique, the location of RSV in midgut, egg and ovary of SBPH was determined. It was found that a large amount of virus granules amassed in follicle cell of ovary, and high density immunogold granules labeled in chorion, egg and midgut cell. The polyclonal antibody was directly prepared from virus granules, thus the results of subcellular location with colloidal gold directly reflected the loci of virus granules in SBPH. The traces of colloidal gold labeling found in chorion and egg has provided direct histochemical evidence that SBPH possesses transovarial transmission ability. Chorion is a non-cellular structural protein, formed by accumulated protein secreted from follicle cell (around the oocyte) in late period of egg formation. It cannot be initiatively acquired virus by endocytic way, so there is only one probability: the virus granules in follicle cell get into chorion along with the secretion of chorionin by follicle cell. Since the early period of vitellus formation, follicle cell has already provided exogenous vitellus for oocyte, it can be suggested that as early as this period, the virus granule in follicle cell has got into egg cell, following with the movement of nutritional materials. However, it cannot rule out another possibility that the oocyte can also directly absorb exogenous vitellus from haemocoel. The following diagram can illustrate the suggested way of transovarial transmission of RSV in SBPH.

5.4 Detection and analysis of the interactions among correlated proteins in high-viruliferous SBPH

Five proteins, namely, encoding coat protein (CP) of RSV, specific protein (SP) of RS disease and three non-structural proteins: NS_2, NS_3, $NSVC_4$, in high-viruliferous SBPH were detected with ELISA and western blotting. Meanwhile, the interactions between CP of RSV and other four proteins in SBPH were also detected[30]. The results showed that in high-viruliferous SBPH, the content of CP was the highest, while the contents of SP, NS_2, NS_3, and $NSVC_4$ successively decreased; and it was also confirmed that there were interactions between CP and SP/$NSVC_4$. These results were consistent with the conclusion mentioned above that SBPH is the host insect of RSV, and RSV can propagate in SBPH. The differences among the contents of the five proteins imply that possibly certain mutually regulatory relationships among these proteins exist in this insect vector. So far, no direct evidence of the hypothesis: "the endogenous bacterium such as *Wolbachia* sp. is related to transmission of virus" proposed by some researchers has been found in this study.

This achievement has won a Second Class Provincial Award of "Progress in Science and Technology" in 2008, a Second Class Ministerial Award of "Bumper Harvest in Agriculture, Animal Husbandry and Fishery"; got one national practical patent, and four national invention patents had passed through examination. One National Trade Standard and one Provincial Locality Standard were drawn up.

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