doi: 10.3969/j.issn.1674-0858.2009.04.004

Genetic diversity of populations of *Harmonia axyridis* (Coleoptera: Coccinellidae) from USA and China revealed by ISSR - PCR

LI Jia-Hui[1], GRENIER Simon[2], SU Zhi-Hui[1,3,4], PANG Hong[1]* (1. State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China; 2. UMR INRA/INSA de Lyon, Biologie Fonctionnelle Insectes *et* Interactions, INRA Villeurbanne, France; 3. JT Biohistory Research Hall, Takatsuki, Osaka 569 - 1125, Japan; 4. Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka 560 - 0043, Japan)

Abstract: The multicoloured Asian ladybeetle, *Harmonia axyridis* (Pallas), is a well-known aphid predator in its native Asian range and appears a threat to native species after imported into America and Europe. In this paper eight different ISSR primers have been used for analyzing population differentiation and genetic variations among one introduced population in America and two native Chinese populations. The resulting 105 loci showed a total polymorphism of 96.19%. Genetic diversity estimated by Nei's gene diversity (*He*), Shannon's information index (*I*) and the percentage of polymorphism loci are similar in native and introduced populations. There is no obvious differentiation estimated by Nei's index (G_{st} = 0.0432) among populations. Most of the genetic variation is observed within populations (AMOVA: 91.22% and mean polymorphism of 94%) and very high rates of gene flow were detected (N_m = 5.537) among populations. Phylogenetic dendrogram constructed based on Nei's genetic diversities suggested that ISSR is a quick and effective technique to study the relationship of geographic populations of *H. axyridis*.

Key words: genetic diversity; *Harmonia axyridis*; inter simple sequence repeat; population analysis; Coccinellidae

异色瓢虫不同地理种群的 ISSR 种群多样性分析

李加慧[1], GRENIER Simon[2], 苏智慧[1,3,4], 庞 虹[1]*

(1. 中山大学有害生物控制与资源利用国家重点实验室,广州 510275; 2. UMR INRA/INSA de Lyon, Biologie Fonctionnelle Insectes *et* Interactions, France; 3. JT Biohistory Research Hall, Takatsuki, Osaka 569 - 1125, Japan; 4. Department of Biological Sciences, Graduate School of Science, Osaka University, Osaka 560 - 0043, Japan)

摘要: 异色瓢虫 *Harmonia axyridis* (Pallas) 在亚洲地区是一种重要的捕食性天敌昆虫,但是当引入到欧美后,对当地的物种产生了威胁。本文用 ISSR 技术对两个中国本地种群和一个引入美国的种群进行了种群多样性以及种群分化的分析。8 条 ISSR 引物共扩增出 105 条带,其中多态性条带比率为 96.19%。Nei's 多样性指数、Shannon 信息指数、多态性条带比率,在中国种群与美国种群中均非常接近,种群分化不明显(G_{st} = 0.042)。AMOVA 分析显示种群多样性主要来源于种群内部,种群间的基因流非常强(N_m = 5.537)。基于 Nei's 多样性构建的聚类结果显示 ISSR 是一种快

基金项目:国家自然科学基金面上项目(30770266)

作者简介:李加慧,男,1984 年生,山东人, E-mail: Jiahui.li@csiro.au

* 通讯作者 Author for correspondence, E-mail: Lsshpang@mail.sysu.edu.cn

收稿时间 Received: 2009 - 06 - 28; 接受日期 Accepted: 2009 - 08 - 10

万方数据

速有效的研究异色瓢虫种群多样性关系的技术。

关键词：遗传多样性；异色瓢虫；ISSR；种群分析；瓢虫科
中图分类号：Q963 文献标识码：A 文章编号：1674-0858(2009)04-0311-09

The multicoloured Asian ladybeetle, *Harmonia axyridis* (Pallas), is a well - known aphid predator in its native Asian region. In the last century it was introduced into Europe (Garcia, 1986) and America (Gordon, 1985) for biological control and has been colonized since the 1990s in North America (Gordon and Vandenberg, 1991; Koch, 2003). Now it appears to be a threat to native ladybird beetle species in some areas (Michaud, 2002; Adriaens *et al.*, 2003; Majerus *et al.*, 2006). Thus, *H. axyridis* gained attention both as a biological control agent and as an invasive species. It also has attracted attention from geneticists because of the extraordinary variation in its wing colour patterns.

Numerous studies on the diversity of *H. axyridis* have been carried out on morphological characteristics based on its color and spotting patterns especially in Asia. One hundred and five types of variations have been reported in the populations according to the study of morphology (Korshefshy, 1932) and five polymorphic types were recognized according to the study of genetics on the color pattern of the beetle forewings (Geng and Tan, 1980). How about it on the molecular level: Does genetic variation exist among populations of *H. axyridis*? Does genetic differentiation occur between populations, and in particular, between native and introduced populations? In this study, we attempt to use the Inter Simple Sequence Repeat (ISSR) markers as a tool to investigate these problems.

It is known that ISSR, consisting of tandemly arranged repeats of several nucleotides, are distributed throughout the genome of organisms and are flanked by highly conserved sequences (Chambers and MacAvoy, 2000). ISSR evolves rapidly and consequently generates a large number of polymorphism. Therefore, the ISSR - PCR method detecting the ISSR - polymorphism developed by Zietkiewicz (1994) and is widely applied to analyze genetic variations at the intra specific level (Wolfe *et al.*, 1998b; Zietkiewicz *et al.*, 1994). Indeed, ISSR - PCR has already been used for assessing hybridization in natural populations (Wolfe *et al.*, 1998a; Wolfe *et al.*, 1998b), for analysis of genetic relationships between or within species (Chatterjee *et al.*, 2004; Vijayan *et al.*, 2006; Wang and Shen, 2007), and for investigating genetic diversity and genetic structure (Angelone *et al.*, 2007; Zhang *et al.*, 2006). ISSR - PCR is a relatively new technique and is considered to provide highly reproducible results compared to other similar methods such as random amplified polymorphic DNA (RAPD) (Takatsuka, 2007), because it produces a large number of PCR fragments with abundant ISSR - polymorphism. In addition, the method is comparatively inexpensive, rapid, and technically simpler than many other molecular marker systems for investigation of genetic instabilities. In this paper, we estimate the genetic diversity and gene flow in three populations of *H. axyridis* using ISSR - PCR.

1 Materials and methods

1.1 Insect materials

Three populations of *H. axyridis* were sampled, including two native populations from the Chinese mainland and one introduced population from the USA. Each population sample consisted of more than 20 individuals. Detailed collection information is shown in Table 1. *H. axyridis* showed a great diversity in color and pattern in our collected samples. Both red and black morphs were collected respectively in the 3 populations.

万方数据

Table 1 Collection information of the three populations of *H. axyridis*

	No. of individuals	Collection site	Collection date
Population C	22	Hunan Province, China	Jun of 2004
Population E	35	Shandong Province, China	Oct of 2005
Population F	24	California, USA	Sep of 2005

1.2 DNA extraction

Total genomic DNA was isolated from each adult individual with universal genomic DNA extraction Kit (TaKaRa) according to the manufacturer's protocol. An absorbance ratio of 260 nm to 280 nm was computed and the quality of the genomic DNA was confirmed. Final DNA samples were stored at −20°C until it was used for downstream applications.

1.3 ISSR - PCR and electrophoresis

Eighty - two ISSR primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. according to the sequences released by University of British Columbia (http://www.michaelsmith.ubc.ca/services/NAPS/Primer_Sets/). A series of preliminary experiments was performed to determine PCR reaction conditions, including temperature for each step of reaction cycle, number of cycles, and quantity of primer and template DNA. Eighty - two primers were initially screened in the preliminary experiments and only eight primers, which gave clear banding pattern, were selected for the final analysis. The sequence of the primers and their annealing temperature (T_m) are listed in Table 2. The reaction mixture (10 uL) contained 0.5 U Taq DNA polymerase, 1.5 mM $MgCl_2$, 2.5×10^{-3} mM dNTP and 30 ng template DNA. PCRs were carried out on PTC150 Thermocycler (MJ Research). The cycling conditions were: initial denaturing at 94℃ for 5 min; 40 cycles each consisting of denaturing at 94℃ for 1 min, annealing at their own T_m for 50 s, extension at 72℃ for 1 min; a single extension at 72℃ for 10 min followed by 4℃ storage.

Table 2 The sequences and their annealing temperature of each selected primer

Serial No. of primers	Primer sequences (5'→ 3')	T_m(℃)
UBC809	AGA GAG AGA GAG AGA GG	48.7
UBC820	GTG TGT GTG TGT GTG TC	49.5
UBC834	AGA GAG AGA GAG AGA GYT	50.0
UBC835	AGA GAG AGA GAG AGA GYC	48.7
UBC840	GAG AGA GAG AGA GAG AYT	49.5
UBC846	CAC ACA CAC ACA CAC ART	48.7
UBC855	ACA CAC ACA CAC ACA CYT	50.5
UBC857	ACA CAC ACA CAC ACA CYG	49.5

The PCR amplification products were separated on 1.6% agarose gels, stained with ethidium bromide, visualized under UV light and photographed.

1.4 Data analysis

Compared with 100 bp ladder marker (MBI Fermentas, USA), reproducible bands of ISSR were manually scored as present (1) and absent (0), and matrices generated by each primer were assembled. From the binary matrix, an unrooted Neighbour - Joining tree based on mean distance was constructed using PAUP version 4.0b10 (Swofford, 2002) to research the relationship of all the individuals at the species level. Analysis of genetic variation among and within populations was performed with POPGEN, including percentage of polymorphic loci, observed number of alleles (*Na*), effective number of alleles (*Ne*), Nei's [1973] gene diversity (*He*), Shannon's information index (*I*), total gene diversity (H_t), gene diversity within populations (H_s), gene diversity between populations (D_{st}), gene flow (N_m). Similarity based Nei's distance between populations were presented in the form of the dendrogram, developed following unweighted pair group method with arithmetic mean algorithm (UPGMA) using SAHN cluster analysis of NTSYS -

万方数据

pc version 2.0 (Rohlf, 1998). Analysis of population structure was performed by AMOVA 1.55.

2 Results and discussion

2.1 ISSR profile

In the present study, eighty - two primers were initially tested. Among them only eight primers produced clear, polymorphic and reproducible fragments. A representative agarose gel was shown by Fig. 1. All of 8 primers were bi - nucleotide ISSR primers, indicating that the genome of *H. axyridis* was rich in such 2 - bases repeat microsatellite. From the electrophoresis results of the PCR products amplified by the eight primers, a total of 105 loci were found from the 3 populations and 101 (96.19%) of them were polymorphic. Within the populations, the number of polymorphic loci were found to be 97 (92.38%), 101 (96.19%) and (82.86%) for populations C, E, and F, respectively (Table 3).

Table 3 Polymorphism loci of the three populations of *H. axyridis*

Population	No. of polymorphic loci	Polymorphic loci*a (%)
Pop C	97	92.38
Pop E	101	96.19
Pop F	87	82.86
Total	101	96.19

*a ISSR - PCR produced a total of 105 loci from the 3 populations.

2.2 Population classification and cluster analysis

An unrooted phylogenetic tree of the 81 individuals of *H. axyridis* was constructed based on mean distance using Neighbour - Joining method on PAUP Version 4 beta 10 (Fig. 2). The tree showed that the 81 individuals are separated into three main groups which are roughly consistent with the three *H. axyridis* populations. The USA population formed a well - clustered group with only one exception (f22). The other two groups were respectively constructed by population C and population E with a few exceptions including e01, e02, e03, e32 and e38. Overall, the individuals of each population were essentially clustered well. This result suggests that each population formed its own distinct lineage, in other words, genetic differentiation has probably occurred between these three populations.

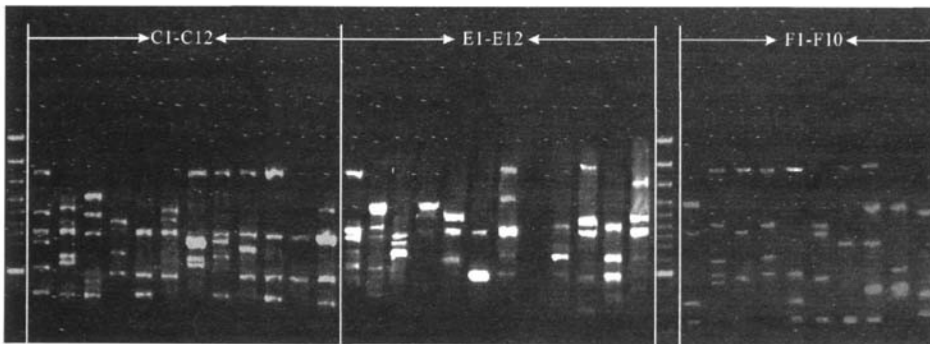

Fig. 1 A representative agarose gel where PCR products amplified by primer 857 were run

To know the genetic relationships of the three populations, we furthermore constructed the UPGMA dendrogram of the three populations based on Nei's genetic distance using SAHN cluster analysis of NTSYS - pc version 2.0. The result showed that the two Chinese populations are closely related, and the USA population is their outgroup (Fig. 3), namely the two Chinese populations have smaller differentiation compared with the US population. As mentioned in introduction, *H. axyridis* was introduced into America in the last

万方数据

century. Despite it having been colonized in the US only for several decades, slightly larger genetic differentiation was observed between the US population and Chinese populations than between the two Chinese populations, probably because of the complete geographical isolation. This result, alternatively, indicates that gene flow may have occurred between the two Chinese populations, which leads to their low genetic differentiation.

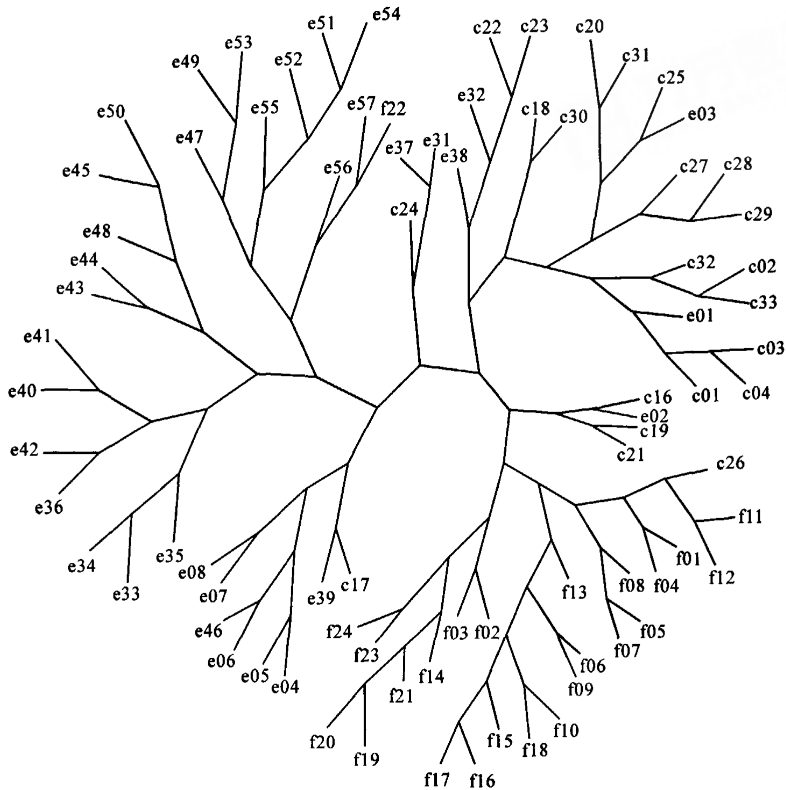

Fig. 2 Unrooted NJ phylogenetic tree of 81 individuals of *H. axyridis* based on mean distance. The individual name indicates its population (e, Shandong, China; c, Hunan, China; f, California, USA) and number

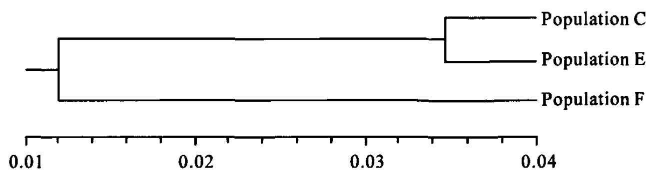

Fig. 3 UPGMA dendrogram based on Nei's genetic distances among the populations of *H. axyridis*

2.3 Population genetic diversity and differentiation

Analysis results of genetic diversity among and within populations are shown in Tables 4 and 5. Nei's gene diversity (or heterozygosity: *He*), Shannon's information index (*I*) and the percentage of polymorphic loci of US population all were the lowest among the three populations (Tables 3 and 4). This is a reasonable result which is most probably attributable to the bottleneck effect caused by the artificial migration of a small part of the native population of *H. axyridis*. On the other hand, the gene diversity and Shannon's infor-

万方数据

mation index within the US population was estimated to be 0.2596 and 0.3968, respectively, which are not largely different from those of the other two native populations (Table 4). There could be two interpretations for this result: 1) the origin of US population shared the majority of the genetic diversity in the native population; 2) US population has quickly increased its genetic diversity accompanied by a rapid expansion of its distribution range in the new habitat. It is generally considered that successful adaptation to the particular habitats where natural enemies exist requires a broad genetic background, namely high genetic diversity in the species gene pool. Indeed, the present study revealed a relatively high level (0.2812 ± 0.0150) of the genetic diversity within populations of *H. axyridis* (Table 5), compared with the estimates in other Coccinellids which is 0.1910 ± 0.0340 (Krafsur *et al.*, 1995; Krafsur *et al.*, 1996; Krafsur *et al.*, 1997). It could be a potential good factor for its quick adaptation to a new habitat, as seen in the fact that *H. axyridis* succeeded in adapting to America conditions in a short period. This finding is also consistent with the wide distribution range of *H. axyridis*.

Table 4 Diversity analysis of the three populations of *H. axyridis* (Mean ± *SD*)

Population	*Na*	*Ne*	*He*	*I*
PopC	1.9328 ± 0.1456	1.4808 ± 0.2993	0.2961 ± 0.1456	0.4543 ± 0.1925
PopE	1.9619 ± 0.1923	1.4201 ± 0.2633	0.2714 ± 0.1350	0.4268 ± 0.1785
PopF	1.8286 ± 0.3787	1.4268 ± 0.3332	0.2596 ± 0.1739	0.3968 ± 0.2411
Total	1.9619 ± 0.1923	1.4615 ± 0.2672	0.2924 ± 0.1310	0.4544 ± 0.1688

A species often consists of some geographical structured populations and in some case genetic (or geographical) differentiation is seen among them. The occurrence of genetic differentiation between populations is considered to be caused by the equilibrium between genetic drift and gene flow, and natural selection by different environmental factors. To know how much the genetic differentiation has taken place between the three populations of *H. axyridis*, Nei's analysis of gene diversity was done in this study. As shown in Table 5, the total gene diversity (H_t) was estimated to be 0.2939 ± 0.0175, which is a little higher than that (0.2812 ± 0.0150) within populations, then the gene diversity between populations (D_{st}) and coefficient of gene differentiation were calculated to be 0.0127 ± 0.0025 and 0.0432, respectively (Table 5). These results suggest that some genetic differentiation occurred between the populations as suggested by phylogenetic analysis above, because D_{st} value was not zero. However, the differentiation degree is extremely low.

The analysis of molecular variance (AMOVA) for 81 individuals in 3 populations of *H. axyridis* showed 91.22% genetic variation ($P < 0.002$) within populations, and 8.78% variation ($P < 0.002$) among populations. This result is consistent with that of the Nei's analysis, indicating that most of the genetic variations of *H. axyridis* existed within populations.

Table 5 Nei's analysis of gene diversity among populations of *H. axyridis*

Parameters	Mean ± SD
Total gene diversity (H_t)	0.2939 ± 0.0175
Gene diversity within population (H_s)	0.2812 ± 0.0150
Gene diversity between populations (D_{st} *b)	0.0127 ± 0.0025
Coefficient of gene differentiation (G_{st} *c)	0.0432
Gene flow (N_m *d)	5.537

*b $D_{st} = H_t - H_s$; *c $G_{st} = D_{st}/H_t$; *d $N_m = (1/G_{st} - 1)/4$

Based on the coefficient of gene differentiation (G_{st}) by Nei's analysis, we obtained a very high N_m value of 5.537 (Table 5), for the case in this study Nm indicates the effective gene flow per generation, namely result showed that there exists very strong gene flow among the populations of *H. axyridis*. Such strong gene flow could effectively restrict the genetic differentiation of *H. axyridis* populations. The introduced US population is geographically isolated from the native Chinese populations, between which natural gene flow can not take place. Thus the gene flow observed in this

万方数据

analysis should be occur almost between Chinese populations. Gene flow is generally thought to result from some migration, or movement of individuals among populations. For *H. axyridis*, the high rate of gene flow may be attributed to the following factors. First, annual migration of *H. axyridis* may be involved. Adult beetles of *H. axyridis* migrating in late autumn and forming aggregation for hibernation have been described in several parts of the world (Koch, 2003; Obata, 1986). Second, humans may be playing an important role in the process of exchange of genetic information. *H. axyridis* was treated as an important bio - control agent and many populations have been reared in the laboratory and released to the farmlands worldwide. This process may destroy their spatial genetic structure, and generate a new genetic structure, leading to low genetic differentiation among the populations. Additional analyses of other populations including the introduced Europe population and other Asian populations could uncover further the population genetic structure of *H. axyridis* in the world.

From these genetic analyses on populations of *H. axyridis*, we can conclude that: (1) A high level of genetic diversity of *H. axyridis* is revealed. (2) Genetic diversity mostly exists within populations and high rate of gene flow were detected among populations, which result in the low genetic differentiation between populations of *H. axyridis*. The high level of intraspecific genetic diversity may be a potential factor for the quick adaptation to a new habitat and rapid expansion of its distribution range. Finally, our results also showed that ISSR is a simple, quick and inexpensive method to study the population genetic structure although it has some technical constraint.

Acknowledgments: We thank Dr. Fenglong Jia and Dr. Suhua Shi for the advice in experimental design, Kebo Deng for beetle collections. Thanks Agnes Vallier, Wei Wu, Suisui Dong and Shulin Deng in the laboratory. Thanks Dr. Ainsley Seago for English correction. The study was supported by grant from the National Natural Science Foundation of China [30770266].

参 考 文 献 (References)

Adriaens T, Branquart E, Maes D, 2003. The multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? *Belg. J. Zool.*, 133: 201 - 287.

Angelone S, Hilfiker K, Holderegger R, Bergamini A, Hoebee SE, 2007. Regional population dynamics define the local genetic structure in *Sorbus torminalis*. *Mol. Ecol.*, 16: 1291 - 1301.

Chambers GK, MacAvoy ES, 2000. Microsatellites: consensus and controversy. *Comp. Biochem. Physiol. B.*, 126: 455 - 476.

Chatterjee SN, Vijayan K, Roy GC, Nair CV, 2004. ISSR profiling of genetic variability in the ecotypes of *Antheraea mylitta* Drury, the tropical tasar silkworm. *Genetika*, 40(2): 210 - 217.

Geng ZC, Tan JZ, 1980. On some genetic problems in the asiatic lady - bird beetle, *Harmonia axyridis* Pallas. *Nature Journal China*, 3 (7): 512 - 528. [庚镇城,谈家桢,1980. 异色瓢虫的几个遗传学问题. 自然杂志, 3(7):512 - 528]

Gordon RD, 1985. The Coccinellidae (Coleoptera) of America North of Mexico. *J. New York Entomol. Soc.*, 93: 1 - 912.

Gordon RD, Vandenberg N, 1991. Field guide to recently introduced species of Coccinellidae (Coleoptera) in North America, with a revised key to North American genera of coccinellini. *Proc. Entomol. Soc Wash.*, 93: 845 - 864.

Koch RL, 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non - target impacts. *J. Insect. Sci.*, 3: 32 - 47.

Korshefshy R, 1932. Coleopterorum Catalogues. Berlin: W Junk.

Krafsur ES, Kring TJ, Miller JC, Nariboli P, Obrycki JJ, Ruberson JR, Schaefer PW, 1997. Gene flow in the exotic colonizing ladybeetle *Harmonia axyridis* in North America. *Biological Control*, 8: 207 - 214.

Krafsur ES, Nariboli P, Obrycki JJ, 1996. Gene flow and diversity at allozyme loci in the twospotted lady beetle (Coleoptera: Coccinellidae). *Annals. Entomol. Society Amer.*, 89:410 - 419.

Krafsur ES, Obrycki JJ, Schaefer PW, 1995. Genetic heterozygosity and gene flow in *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae). *Biological Control*, 5: 104 - 111.

Majerus M, Strawson V, Roy H, 2006. The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. *Ecological Entomology*, 31: 207 - 215.

Michaud JP, 2002. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, *Cycloneda sanguinea*. *Environ. Entomology*, 31: 827 - 835.

Obata S, 1986. Determination of hibernation site in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae). *Japanese Journal of Entomology*, 54: 218 - 223.

Rohlf FJ, 1998. NTSYS - pc: Numerical Taxonomy and Multivariate Analysis System, version 2.0. Setauket, NY: Exeter Software.

Swofford DL, 2002. PAUP*: Phylogenetic analysis using parsimony (* and other methods) Version.

Vijayan K, Anuradha HJ, Nair CV, Pradeep AR, Awasthi AK, Saratchandra B, Rahman SAS, Singh KC, Chakraborti R, Urs SR, 2006. Genetic diversity and differentiation among populations of the

万方数据

Indian eri silkworm, *Samia cynthia ricini*, revealed by ISSR markers. *J. Insect. Sci.*, 6: 30.

Wang XY, Shen ZR. 2007. Potency of some novel insecticides at various environmental temperatures on *Myzus persicae*. *Phytoparasitica*, 35: 414-422.

Wolfe AD, Xiang QY, Kephart SR, 1998a. Assessing hybridization in natural populations of Penstemon (Scrophulariaceae) using hypervariable intersimple sequence repeat(ISSR) bands. *Mol. Ecol.*, 7: 1107-1125.

Wolfe AD, Xiang QY, Kephart SR, 1998b. Diploid hybrid speciation in Penstemon (Scrophulariaceae). *Proc. Natl. Acad. Sci. USA*, 95: 5112-5115.

Zhang XP, Li XH, Qiu YX, 2006. Genetic diversity of the endangered species *Kirengeshoma palmata* (Saxifragaceae) in China. *Biochemical Systematics and Ecology*, 34:38-47.

Zietkiewicz E, Rafalski A, Labuda D, 1994. Genome fingerprinting by simple sequence repeat (SSR) - anchored polymerase chain reaction amplification. *Genomics*, 20: 176-183.

Appendix 1 Data matrix produced by ISSR-PCR

Individuals	Primer 809	Primer 820	Primer 8	Primer 834	Primer 835	Primer 840	Primer 855	Primer 857
c01	0000000110111	001000100100	100101111111	0101111110100	0001010010000	101110	01101100010101010001	101000011001000
c02	0000110110000	000001100110	000110101111	01001110001110	0111001001010	101011	01000101100001110011	101100111101001
c03	0010000110011	000001100000	100101111111	01101111001110	0000001001001	101111	00100011010011100011	000001111110000
c04	0000000100000	000001100100	000101110111	10001111011111	0011001110101	100011	00100010010001100001	101000100100100
c16	0010100101010	001001100010	001000001111	10000110100000	0101011010110	001011	01010010010011110001	001000100011001
c17	0000100000100	000010000110	001010110111	01001111000011	0000011110000	011011	00001010000101100011	011011101001110
c18	0000000100110	010000000010	000000000011	11110110111110	0000000110000	001011	01101011010001100111	110001100111000
c19	0010001000010	110000000000	001000000010	00001011101101	0000000000100	010011	01010000110001000111	001101000101110
c20	0000010100111	000000001110	000000001111	10001010101110	0001111101001	111010	01000100100111100111	010010100111000
c21	0111100001001	001000100010	000010000010	00000010100110	0001000100011	001011	01000100100001000111	000001110101100
c22	1000000011011	000100001010	101000001010	10100111100110	0000101110001	001111	00001100100101100111	111010000001100
c23	1001100011011	100010001110	000100000010	10001011010110	0100011100010	101111	01011010100101100101	010010010101111
c24	0100011010111	010001000110	001011000110	00001111101110	1000000010000	001011	00010101010101101001	100100110010010
c25	0100001010011	100010100010	001001011111	10101110011100	0010110101001	111010	00000101010001001011	111010101101010
c26	0100100010010	000001000100	000011111010	10001110011100	1000001000010	001010	00010000010001011011	010100101001111
c27	0000100110111	001000101110	000010000110	00001110011100	0000001000000	101111	10001101011001100011	000011001101000
c28	0000011010011	000001100000	000100011110	10000110011100	0000011101000	101011	01001101000101100011	000011101011001
c29	0000000010011	000001101010	000100001110	10000111001010	0010011011000	011111	10000101010101100011	001011101101000
c30	0000010010011	000010100010	000000110010	10001110011110	0000100111000	001111	01111101000001100001	101000110110100
c31	0000010001111	000100100110	001000011011	10010110100100	0000010101000	111111	10001100000011000111	110110110110001
c32	0000010010110	000000101010	001100111011	10010110101110	0100011000101	001011	10100101101101000011	100010111001100
c33	0000001010010	000010100100	101000101111	01100110011010	0100011001001	101111	00100100100001110011	100000101001001
e01	1010000000111	000010001100	100100101111	00011110111110	0110000010010	101111	10000100010001100111	100001100000100
e02	0000001010111	000101000100	000000101111	00000110010111	0000100100110	001011	01000101010001100111	001000101011001
e03	0111001010111	010100100110	001010011111	10001110000100	0000010000000	111010	00101001010101100011	100011101111100
e04	0000010010110	000010001101	101010111111	00111110000100	0000010001000	100011	01000111000101000001	101001101111111
e05	0000001010010	000010000001	100011111111	00011110000100	0001010011000	011010	01011001000111100001	100010101111100
e06	0101101010010	000111101001	000000110010	00000110000100	0101110000010	111010	10010001000011100001	010101000111100
e07	0000001000011	000100100100	001000001011	00000111000101	0000010001000	001111	00001001000111000001	000101001101101
e08	0000010110010	000011101110	001010010111	00001111000101	1001010000000	111111	01001000000101100011	000000000111001
e31	1001011010011	000100100010	000000001011	00101111010100	0010000110010	101111	00100110010101000001	110001001001000
e32	0000000101010	000010001011	001000101010	00101110000101	0000001100000	001110	01000000000111110001	010010001100000
e33	0000110111011	000010001011	000111010010	00001110000100	0000000000010	011010	10001101010001000001	100001111001000
e34	0000110111011	000010101011	000111011010	00001110000100	1001100000000	011010	10001101010001000001	100011000101000
e35	0000110001011	000001001001	000101000010	00001110000100	0001000111001	011010	01000000010001100001	000101101001001
e36	0001111001011	000001000011	100011010010	11101111000100	1100000000110	001110	00000100000101000001	000101101001001
e37	0000111011010	000000101111	101110000110	10001110010100	1000001001000	100111	00100111010101100001	101100110100110

万方数据

(续上表)

Individuals	Primer 809	Primer 820	Primer 8	Primer 834	Primer 835	Primer 840	Primer 855	Primer 857
e38	0000011101010	000000101111	100010000111	11001110010100	1001010010000	101111	00000010000111000011	100000110001110
e39	0000000000010	001010001101	000000000011	00000010000101	0000001000000	000010	00011111000011000011	001001100000110
e40	0000011000011	000000101011	100110000010	10101010000100	0000010000000	111110	00101001010001100011	000000111001000
e41	0000011001011	000001000001	100110011010	11111010000101	0000000001100	111010	00101001010011000011	001000110100000
e42	0001111001010	000001001001	110110010010	10100010000100	0000001010000	101011	01000110010101000011	000011001101001
e43	1000011001011	000001001001	000000010011	00000111100000	0001000010001	001111	00100001000001011101	101010100000100
e44	0001111001110	000000001001	000110010011	00001010100010	0000000001000	011110	00100001000001001101	010001100100100
e45	0100001000110	000000011001	100011100010	00000110000100	0010001000001	011110	00110000000001000001	010000100001000
e46	0000110010110	000001001001	000110110110	00100110000100	0000011001000	010010	00100101010101100001	001011010100100
e47	0101000001010	000000001101	001111100011	01010110101100	0100001010101	001110	10000101010111101111	111000000000001
e48	0100011001110	000000001101	000100001001	00001010000100	0000111000000	101110	10001000000001000001	100001110101000
e49	1001010011111	000100101101	010010010011	01001110001100	0110011010000	001011	10100001000101100011	101101000001000
e50	0000011011110	100000010001	000001100110	01011010000100	0000110000001	101011	00100000000001000001	110011000001010
e51	1000100001001	000000010001	000010101011	00011011100100	0010010010011	001111	10100011010011101011	001010001100010
e52	0001000011010	000000111001	010001100010	00001011001100	0000101010011	001111	10100010101001100011	100000100100001
e53	1001010010010	000100110101	001000011011	00011010001100	0000011010111	001011	10100110000111100001	010100100001010
e54	0000100011011	000100110101	000101101011	00000111100000	0000111010111	101111	10101111010001100001	001000000100000
e55	0001110001011	000010100110	010011100010	10001110001100	1000110010100	101111	00100010010001001011	010000000100101
e56	0000100001010	000010100110	000010000011	10000011001100	0000001001000	111010	00100010010001100111	100000011001100
e57	0001110001101	100010000100	000111011110	00000111001100	0000100000001	011011	11000010010001110011	000000001001001
f01	0000010000011	001010000110	000001000111	10011110110100	0000000100011	001011	01000000010001111001	010001000001101
f02	1001110011011	000000010110	000000110110	00100110101100	0000100100011	100111	01000010000101101011	000001001101110
f03	0001110000011	110100010110	000000010110	10100110110100	0010111100110	000111	01000000010001100001	000000101101011
f04	0000000011011	001101001110	000010011111	10101110111100	0000101000001	101010	01100100010001110011	010001101001101
f05	1000100011001	000101000110	000011000111	10100110110100	0001001011001	011111	01100101110101100011	000100000000000
f06	0010110011001	000010000110	000110011111	10101110110100	0000101000001	001111	01111101110101111011	000101110101101
f07	1000100011001	000001000110	000110000111	10101110110100	0010101000001	001010	01001101110001101011	110000001001010
f08	0000100011010	100111000010	000010100111	10001110100100	0010101000000	110111	01100100110101100011	111000000001010
f09	0000001010111	000010000110	000000011011	10000110110100	0001001100000	001011	01011110010101101011	000101110100110
f10	0000100011011	011000000001	010010010110	10000110110100	0000001000000	000011	00001101010101101011	000001100101011
f11	1000100011000	000000010111	100001000111	10001110010100	0001101000010	001111	01000000010111100101	110100010001011
f12	1000100000000	000001010011	000000110010	00001110110100	0000001100000	001110	01010000010001100011	111100011000011
f13	0000000011010	000000010001	000001001110	10101110110100	0001101110000	101111	01000101010001111011	001000110010100
f14	1000100000010	100000010001	000000010110	10101110101100	0001001010011	101010	01010000010101100001	000001100110101
f15	0010100011011	000010010000	010100001111	00100110101100	0000001010010	001011	01000101000001101011	100000111001111
f16	0010101010000	110000010101	010010001111	10100110101100	1001101010000	111110	01001100010111100011	100001100111110
f17	0010100010010	000000010010	010100011111	10100110100100	0000100010000	100010	01001101010111100011	100000000011101
f18	0000101001101	011000000011	000110110111	10100110101100	0001001010000	010010	01001111000111101011	000001100000011
f19	1000010010010	001000010011	000001110111	10101110101100	1000001010010	100010	01100011010101101011	100100010000010
f20	1000010010110	100000000011	000101110011	10101110010100	1000001010010	000010	01100001000101101011	101000010000011
f21	1000010000110	100000010011	000000100111	00100110111100	0000000000011	010111	01100001000101100001	001101000010011
f22	1000110001000	000010000000	010111110010	00100110111100	0000001110001	010010	00000101000001100011	000100000000101
f23	1011100010111	101000010101	000011110111	00101110101000	0000011001001	010011	01000101000101100011	000000001010000
f24	1000000010111	001000100101	000100110111	00100110101000	0001001010000	001111	01000101000001100011	000001101100100

万方数据

异色瓢虫不同地理种群的ISSR种群多样性分析

万方数据 WANFANG DATA 文献链接

作者： 李加慧， GRENIER Simon， 苏智慧， 庞虹， LI Jia-Hui， GRENIER Simon， SU Zhi-Hui ， PANG Hong

作者单位： 李加慧,庞虹,LI Jia-Hui,PANG Hong(中山大学有害生物控制与资源利用国家重点实验室,广州,510275)， GRENIER Simon,GRENIER Simon(UMR INRA/INSA de Lyon,Biologie Fonctionnelle Insectes et Interactions,France)， 苏智慧,SU Zhi-Hui(JT Biohistory Research Hall,Takatsuki,Osaka569-1125,Japan;Department of Biological Sciences,Graduate School of Science,Osaka University,Osaka 560-0043,Japan)

刊名： 环境昆虫学报ISTIC PKU

英文刊名： JOURNAL OF ENVIRONMENTAL ENTOMOLOGY

年，卷(期)： 2009，31(4)

被引用次数： 0次

参考文献(23条)

1. Adriaens T.Branquart E.Maes D The multicoloured Asian ladybird Harmonia axyridis Pallas (Coleoptera:Coccinellidae),a threat for n-ative aphid predators in Belgium?[期刊论文]-Belgian Journal of Zoology 2003

2. Angelone S.Hilfiker K.Holderegger R.Bergamini A,Hoebee SE Regional population dynamics define the local genetic structure in Sorbus torminalis[期刊论文]-Molecular Ecology 2007

3. Chambers GK.MacAvoy ES Microsatellites:consensus and controversy[期刊论文]-Comparative Biochemistry and Physiology Part B:Biochemistry and Molecular Biology 2000

4. Chatterjee SN.Vijayan K.Roy GC.Nair CV ISSR profiling of genetic variability in the ecotypes of Antheraea mylitta Drury,the tropical tasar silkworm[期刊论文]-Genetika 2004(02)

5. 庚镇城.谈家桢 异色瓢虫的几个遗传问题[期刊论文]-自然杂志 1980(07)

6. Gordon RD The Coccinellidae (Coleoptera) of America North of Mexico[期刊论文]-Journal of New York Entomological Society 1985

7. Gordon RD.Vandenberg N Field guide to recently introduced species of Coccinellidae (Coleoptera) in North America,with a revised key to North American genera of coccinellini[期刊论文]-Proceedings of the Entomological Society of Washington 1991

8. Koch RL The multicolored Asian lady beetle,Harmonia axyridis:A review of its biology,uses in biological control,and non -target impacts[期刊论文]-Journal of Insect Science 2003

9. Korshefshy R Coleopterorum Catalogues[期刊论文]-Berlin:W Junk 1932

10. Krafsur ES.Kring TJ.Miller JC.Nariboli P Obrycki JJ Ruberson JR Schaefer PW Gene flow in the exotic colonizing ladybeetle Harmonia axyridis in North America[期刊论文]-Biological Control 1997

11. Krafsur ES.Nariboli P.Obrycki JJ Gene flow and diversity at al-lozyme loci in the twospotted lady beetle (Coleoptera:Coccinellidae)[期刊论文]-Annals Entomol Society Amer 1996

12. Krafsur ES.Obrycki JJ.Schaefer PW Genetic heterozygosity and gene flow in Coleomegilla maculata De Geer (Coleoptera:Coccinellidae)[期刊论文]-Biological Control 1995

13. Majerus M.Strawson V.Roy H The potential impacts of the arrival of the harlequin ladybird,Harmonia axyridis (Pallas) (Coleoptera:Coccinellidae),in Britain[期刊论文]-Ecological Entomology 2006

14. Michaud JP Invasion of the Florida citrus ecosystem by Harmonia axyridis

(Coleoptera:Coccinellidae) and asymmetric competition with a native species[期刊论文]-Cycloneda sanguinea Environ Entomology 2002

15.Obata S Determination of hibernation site in the ladybird beetle,Harmonia axyridis Pallas (Coleoptera,Coccinellidae)[期刊论文]-Japanese Journal of Entomology 1986

16.Rohlf FJ NTSYS-pc:Numerical Taxonomy and Multivariate A-nalysis System,version 2.0[期刊论文]-Setauket,N Y:Exeter Software 1998

17.Swofford DL PAUP*:Phylogenetic analysis using parsimony (* and other methods) Version[期刊论文]-2002

18.Vijayan K.Anuradha HJ.Nair CV.Pradeep AR,Awasthi AK,Saratchandra B,Rahman SAS,Singh KC,Chakraborti R,Urs SR Genetic diversity and differentiation among populations of the Indian eri silkworm,Samia cynthla ricini,revealed by ISSR markers[期刊论文]-Journal of Insect Science 2006

19.Wang XY.Shen ZR Potency of some novel insecticides at various environmental temperatures on Myzus persicae[期刊论文]-Phytoparasitica:Israel Journal of Plant Protection Sciences 2007

20.Wolfe AD.Xiang QY.Kephart SR Assessing hybridization in natural populations of Penstemon (Scrophulariaceae) using hyper-variable intersimple sequence repeat (ISSR) bands[期刊论文]-Molecular Ecology 1998

21.Wolfe AD.Xiang QY.Kephart SR Diploid hybrid speciation in Penstemon (Scrophulariaceae)[期刊论文]-Proceedings of the National Academy of Sciences(USA) 1998

22.Zhang XP.Li XH.Qiu YX Genetic diversity of the endangered species Kirengeshoma palmata (Saxifragaceae) in China[期刊论文]-Biochemical Systematics and Ecology 2006

23.Zietkiewicz E.Rafalski A.Labuda D Genome fingerprinting by simple sequence repeat (SSR)-anrhored polymerase chain reaction amplification[期刊论文]-Genomics 1994

相似文献(4条)

1.学位论文 吴庆禹 异色瓢虫不同色斑类型遗传多样性研究 2007

异色瓢虫Harmonia axyridis(Pallas)是一种重要的捕食性天敌昆虫，也是一个典型的多型性物种，它是蚜虫、松干蚧、粉蚧等害虫的主要天敌。现在正被人们广泛释放用来对蚜虫和蚧虫进行生物防治。

本文主要从形态学水平、生态水平、分子水平对异色瓢虫不同色斑类型的遗传多样性进行了研究，揭示出异色瓢虫不同色斑类型间在遗传多样性方面的差异，为深入了解异色瓢虫的生存状况、分类地位、进化历史和亲缘关系，预测其未来的发展趋势，更有效地进行生物综合防治奠定了基础。

(1)对2178只异色瓢虫的斑型进行统计的结果，共发现126种色斑类型，其中有15种色斑型在以前的报道中没有记录。多样性分析的结果，Monk指数、Simpson多样性指数以及 Shannon-Wiener指数分别为0.0579，0.901和1.56。

(2)对黄底型异色瓢虫的斑点的概率进行了统计，其中，斑点 3 的概率最高，而斑点9和斑点1/2的概率则较小。不同的色斑型异色瓢虫间，雌雄比例差别很大；尤其是在黄底型类群中，随着斑点数的增多，类型中雄性比例减小。对异色瓢虫主要的类群之间的体长、体宽进行了比较研究，结果显示在体长和体宽方面差异显著，在长宽比方面差异极显著；说明不同色斑类型间，在虫体的大小和形状上存在一定的差异。

(3)通过扫描电镜的观察，描述了异色瓢虫不同色斑类型触角形态结构，以及触角上感器的种类和数量；在不同色斑型间进行了对比，结果显示异色瓢虫不同色斑类型之间存在一些差异。

(4)异色瓢虫不同色斑类型越冬成虫的生态分布与其活动的林型、栖息植物、发生时间、气候变化等有直接关系。在同一时间同一地域数量和分布寄主上存在一些差异。

(5)异色瓢虫不同色斑类型越冬成虫的空间分布型为聚集分布，并符合负二项分布。

(6)本试验用改良CTAB法、饱和NaCl法以及CTAB法与SDS法相结合3种方法，进行综合设计，探索出一套操作简便、快速、准确的高质量异色瓢虫DNA提取和纯化技术。

(7)从40条RAPD随机引物中筛选出12条具有多态性的引物，对9种异色瓢虫不同色斑类型进行RAPD分析，共检测到多态位点169个，位点范围在100bp～2000bp之间，多态位点百分率在34.32％～65.68％之间；从40个引物中筛选出13条条带清晰且多态性丰富的ISSR引物，对13种异色瓢虫不同色斑类型进行ISSR分析，共扩增出127条ISSR标记带，其中多态性条带120条，多态性百分率94.49％。

(8)RAPD和ISSR分析的一致性和可信度：由RAPD和ISSR分析算得的两组相似系数之间相关性系数r=0.928(n=127)，达到极显著水平。表明应用这两种技术对异色瓢虫遗传多样性与亲缘关系的分析具有较高的一致性和可信度。但是这两种方法的聚类结果在个别色斑型分类上仍存在一定的差异。(9)依据RAPD 分析结果，对供试的9种异色瓢虫不同色斑类型进行聚类分析，结果表明：它们之间的遗传相似系数在0.46～0.58之间；依据ISSR分析结果，对供试的13种异色瓢虫不同色斑类型进行聚类分析，它们之间的遗传相似系数在0.36～0.60之间。

综上所述，通过从形态学水平、生态水平、分子水平对异色瓢虫不同色斑类型的遗传多样性进行比较，异色瓢虫不同色斑类型间在遗传多样性方面存在一定的差异，为人类更好地保护、开发和利用天敌昆虫资源提供了科学依据。

2.期刊论文 关桦楠.迟德富.宇佳.李晓灿.Guan Huanan.Chi Defu.Yu Jia.Li Xiaocan 帽儿山地区异色瓢虫遗传多

样性的ISSR分析 -东北林业大学学报2007,35(9)

利用ISSR技术对东北帽儿山地区13种不同色斑型异色瓢虫进行基因组多态性分析,从40条引物中筛选出13条用于异色瓢虫的ISSR扩增.共扩增出127条带,其中多态性条带120条,多态性百分率为94.49%.根据ISSR扩增结果,利用NTSYSpc2.10e软件进行Jaccard相似系数分析,13种不同色斑型异色瓢虫的遗传相似系数为0.36～0.60,平均相似系数为0.51.通过UPGMA聚类分析,在遗传相似系数0.50处,13份瓢虫材料可分为2大类群.主成分分析结果表明,显明变种、四斑变型和二窗无脊3种斑型瓢虫是异色瓢虫遗传变异的主线.ISSR分析表明,异色瓢虫的遗传多样性较为丰富.笔者还根据聚类结果对瓢虫鞘翅底色变异的可能因素进行了分析.

3.期刊论文 程琳.迟德富.张晟铭.杨爱红.Cheng Lin.Chi Defu.Zhang Shengming.Yang Aihong 帽儿山地区不同色斑型异色瓢虫的SRAP分析 -东北林业大学学报2009,37(5)

利用SRAP技术,从72对引物中筛选出10对引物对帽儿山地区的10种不同色斑型(2种斑型组)异色瓢虫进行扩增,对扩增结果进行基因组多态性分析,结果显示,10对引物共扩增出条带175条,其中多态性条带为133条,多态位点比率为76.04%.利用POPGENE 32软件进行不同斑型组遗传多样性指数的分析,通过非加权配对算术平均法(UPGMA)进行聚类分析,结果表明,帽儿山地区异色瓢虫的遗传多样性较为丰富,部分异色瓢虫的亲缘关系与其底色之间或者色斑形状之间存在一定的关联性,与形态分类结果一致.

4.学位论文 辛海泉 吉林省部分地区异色瓢虫鞘翅色斑多态的群体遗传学分析 2009

异色瓢虫（Harmonia axyridis Pallas）原主要分布于亚洲大陆，而目前在世界大多数地方都能看见它们的踪迹，异色瓢虫是一个典型的多型性物种，以往对其研究主要集中在蚜虫的生物防治上。自从谈家桢揭示了异色瓢虫鞘翅色斑嵌镶显性（mosaic dominance）规律以来，鞘翅色斑遗传多样性问题，久为分类学家、遗传学家及生态学者所注目，其间他们进行了大量的研究工作。有些结果显示了异色瓢虫色斑变异有着时间和地理方面的规律。可能说明不同斑型的异色瓢虫对自然环境的变化的适应性有所不同。

本文是对长春市2007年、2008年在长春市区，双阳区、敦化市等地采集的异色瓢虫数据进行的分析，我们将采集的瓢虫分为四种类型：黄底型（succinea）、二窗型（conspicua）、四窗型（spectabilis）、花斑型（axyridis）。研究和以往各地采集数据进行比较，我们将春天采集没有能够越冬的死亡瓢虫同下个冬天前越冬的活体瓢虫数据进行分析，找出色斑频率的时间变化和地理变化趋势，同时应用回归分析对温度因素与异色瓢虫色斑变异影响的相关性进行了研究。

结果显示长春市区2008年与1989年相比，二窗型瓢虫表型及基因频率增长，黄底型瓢虫表型及基因频率有所下降。异色瓢虫色斑频率变化越冬前后没有变化，各色斑型异色瓢虫越冬能力相差不明显。小范围内，异色瓢虫越冬集群间色斑表型频率和基因频率差异不显著，各色斑瓢虫集群时没有倾向性。异色瓢虫色斑表型频率和基因频率年代变异与年平均温度变化相关性显著。纬度低的地区瓢虫色斑表型频率和基因频率变异波动大于纬度高的地区。

本文链接：http://d.wanfangdata.com.cn/Periodical_kctd200904004.aspx

下载时间：2010年7月15日