西北林学院学报 2010，25(2)：108～112
Journal of Northwest Forestry University

A Preliminary Study on Isozymes in Four Species of Stink Bugs in Qinling Mountainous Region (Hemiptera：Coreidae)

WANG Qi-rui[1]，ZHU Gang-li[2]，ZHENG Zhe-min[3]，
FAN Qin[1]，SUN Xue-gang[1]

(1. *College of Traditional Chinese Medicine，Southern Medical University，Guangdong，Guangzhou* 510515，*China*；2. *Center of Environment Engineering，Guangdong Vocational and Technical School of Environmental Protection，Guangdong，Guangzhou* 510655，*China*；3. *College of Life Science，Shaanxi Normal University，Xi'an，Shaanxi* 710062，*China*)

Abstract：This paper deals with the esterase isozymes of four species of subfamily Rhopaline and Alydinae(Hemiptera：Coreidae) in Qinling Mountainous region by the vertical slab polyacrylamide gel electrophoretic technique. The variances between the two genera of Rhopalinae are obvious than intraspecific differences. There are 4 and 5 loci resolved for *Stictopleurus minutus* and *Riptortus pedestris*，respectively. All the esterase present as monomeric enzyme except for these which were dominated by monomorphic loci. The isozyme polymorphism of individuals within a Coreidae species is results from the pair of different alleles at the same loci. And sex-linked gene may result in the difference between female and male within species. Differences in allic among three populations of *R. pedestric* appeared，but the data available is limited and calls for further research.

Key words：Coreidae；Rhopaline；Alydinae；esterase isozyme；locus；polymorphism
中图分类号：Q969.352.3　**文献标志码**：A　**文章编号**：1001-7461(2010)02-0108-05

秦岭4种缘蝽同工酶的初步研究(半翅目：缘蝽科)

王启瑞[1]，朱刚利[2]，郑哲民[3]，范　钦[1]，孙学刚[1]

(1.南方医科大学 中医药学院，广东 广州 510515；2. 广东省环境保护职业技术学校 环境工程中心，广东 广州 510655；3. 陕西师范大学 生命科学学院，陕西 西安 710062)

摘　要：应用聚丙烯酰胺凝胶电泳技术研究了秦岭4种缘蝽的EST同工酶。研究结果表明，姬缘蝽亚科2属间差异要大于属内各种间差异。控制开环缘蝽及点蜂缘蝽的EST位点分别为4和5个，而且乙酸萘酯为底物的酯酶除未发现杂合子而无法确定的以外，都是单体酶，不同个体间酶谱差异主要是同一位点来自父母本的2个等位基因的不同而造成的，雌雄差异可能由性连锁基因引起。点蜂缘蝽3个居群等位基因频率存在差异，还需要进一步试验研究。

关键词：缘蝽科；姬缘蝽亚科；蛛缘蝽亚科；酯酶同工酶；基因座；多态性

The technique of isozyme electrophoresis is widely used as a valuable method in studying classification of species，identification of genetic relationship[1-2]，genetic expression and regulation in ontogeny[3]. It is also an effective tool in helping to discriminate among species and to reveal genetic structure of population and is particularly useful for analysis of genotypic biodiversity[4]. There have been many researches that covered the application of isozymes to animal taxonomy. However，domestic study on stink bug is scarce[5-7]. And most of the papers about stink bugs before had only studied on several specimens by the number and relative migration rate of enzyme bands scored

收稿日期：2009-07-02　**修回日期**：2009-09-18
作者简介：WANG Qi-rui，Female，Henan Province，phD，lecturer，Molecular Systematics and molecular immunology.

from the gel[7]. Genetics analysis has not been covered yet.

In this paper, we aims to speculate the phylogenetic relationships of four Coreidae species distributed in the region of the Qinling Mountains and analyze genetic variability with large samples. We expect to provide some fundamental data that could be applied in the research of Coreidae molecular systematics and genetic differentiation.

1　Materials and Methods

1.1　Insects

Four Coreidae species, *Stictopleurus minutus* Blöte, *Aeschyntelus bicolor* Hsiao, *A. notatus* Hsiao and *Riptortus pedestris* Fabricius, were collected in diverse locations in Qinling Mountainous region (Table 1). In all cases, taxonomic determination was made by morphological characters following the directions of Xiao[8] *et al*. After hungered for 24 h, all the 155 individuals were directly frozen at −70℃ for later analysis from 17 August, 1999 until 20 April, 2001.

1.2　Esterase isozyme analysis

For this analysis, the esterase isozyme system was analyzed using extracts of the whole individuals prepared. Electrophoretic and scanning procedures were detailed in previous paper of this series. Henceforth 'this series' refers to Wang [6-7] *et al*.

1.3　Clustering methods

Each specimen was characterized by the relative migration rate (R_m) of its bands scored from the gel. When the band was present then wrote down one, when absent then wrote down zero[9-10]. After that, the [0,1] data was processed with the software package Statistical Program for Social Sciences (SPSS).

1.4　Genetic interpretation

The principle of genetic interpretation was based on Wang[9] and Huang[10].

Table 1　Characteristics of different bugs analyzed

表 1　实验蝽象特征

Species	N	Date of collection	Host	Location
Rhopalinae				
Stictopleurus Stål				
S. minutus Blöte	21♂15♀	2000/8/18	*Erigeron annuus* (Linn.)Pers.	Huoditang forest
Aeschyntelus Stål				
A. bicolor Hsiao	1♂1♀	1999/7/16	*Erigeron annuus* (Linn.)Pers.	Nanwutai
A. notatus Hsiao	5♂4♀	2000/6/8	*Erigeron annuus* (Linn.)Pers.	Huoditang forest
Alydinae				
Riptortus Stål				
R. pedestris Fabricius	12♂18♀	1999/7/14	*Zea mays* L.	Cuihua Mountain
	22♂25♀	1999/8/8	*Phaseolus vulgaris* L.	Xunyangba
	16♂15♀	2000/8/10	*Phyllostachys heteocycla* cv. *pubescens*	Louguantai

2　Results

2.1　Esterase isozymes of each species of Rhopalinae

The esterase data presented a more polymorphic phenomenon that there were several zones of enzyme activity with variant in each zone (Fig. 1). All bands scored from the gels could be divided into four regions based on their relative mobility.

EST-I: Relative mobilities ranged from 0.42 to 0.54. There are totally 11 enzyme bands. All the deep brown bands in this region had higher activity.

EST-II: Relative mobilities ranged from 0.35 to 0.46. The 11 deep red bands with stable number and activity all transferred quickly. *A. bicolor* had no band in this region perhaps for long time of refrigeration or other unknown reasons.

EST-III: Relative mobilities ranged from 0.28 to 0.34. Those 3 washy red enzyme bands had low activity.

EST-IV: Relative mobilities ranged from 0.05 to 0.25. The 11 protein bands had larger alteration among the three species.

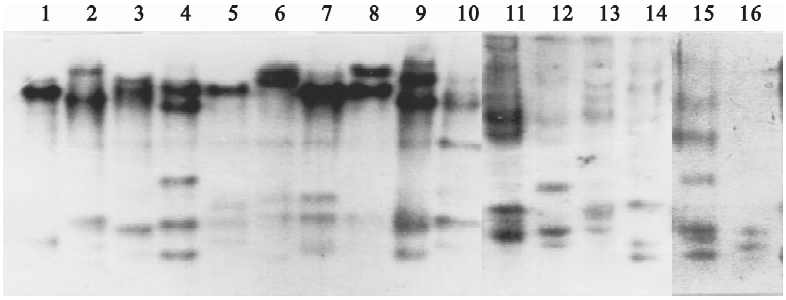
2.2　Esterase isozymes of three populations of *R. pedestris*

All bands scored from the gels could be divided into five regions based on their relative mobili-

ties (Fig. 2).

2.3 Phylogenetic clustering

With reference to the dendrogram (Fig. 3) based on EST isozyme of three species of subfamily Rhopaline, we could see that *A. bicolor* was grouped with *A. notatus*. Euclidean distance between them was 3.240. Euclidean distance between *S. minutus* and the two *Aeschyntelus* species was 3.629.

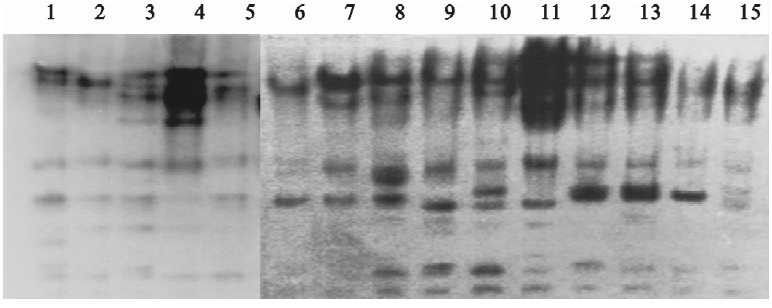


S. minutus:1～10; *A. notatus*:11～14 ; *A. bicolor*: 15～16(All samples are female except for No. 8,9,10,14,16)

开环缘蝽:1～10;点伊缘蝽:11～14;二色伊缘蝽:15～16(8,9,10,14,16 泳道标本为雄性,其余为雌性)

Fig. 1 EST isozyme patterns of three species of subfamily Rhopaline

图 1 姬缘蝽亚科 3 种蝽象 **EST** 同工酶图谱



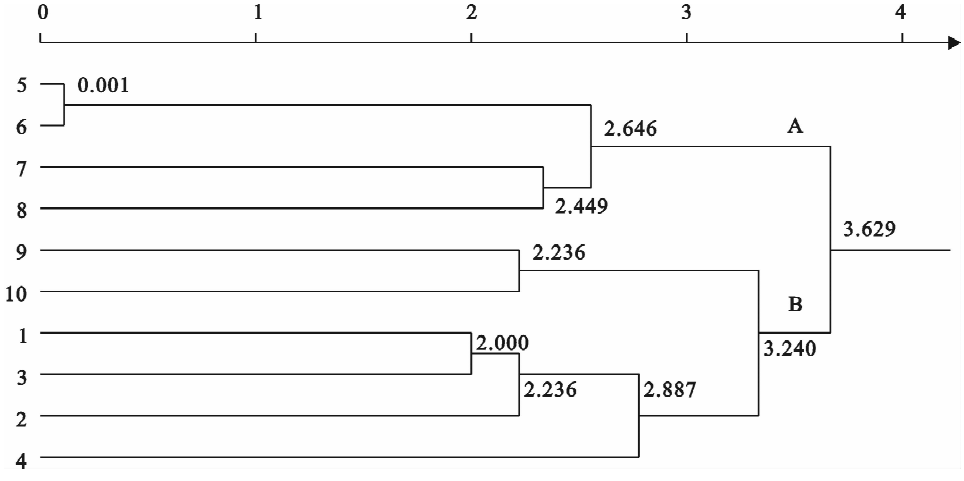
1～5: population from Louguantai 6～9: population from Cuihua Mountion;

10～15:population from Xunyangba (All samples are female except for No. 5,6,7,13,14,15)

1～5:楼观台居群;6～9:翠华山居群;10～15:旬阳坝居群(5,6,7,13,14,15 泳道标本为雄性,其余为雌性)

Fig. 2 The photographs about 3 populations of *R. pedestris* in isozymes electrophoresis

图 2 点峰缘蝽 3 居群 **EST** 同工酶电泳图谱



A. *Stictopleurus* 5～8;*S. minutus*; B. *Aeschyntelus*; 9、10; *A. bicolor*; 1～4; *A. notatus*

(All species are female except for No. 4,7,8,9)

A. 环缘蝽属 5～8:开环缘蝽;B. 伊缘蝽属;9、10:二色伊缘蝽;1～4:点伊缘蝽(4,7,8,9 泳道标本为雄性,其余为雌性)

Fig. 3 The dendrogram based on EST isozyme of three species of subfamily Rhopaline

图 3 姬缘蝽亚科 3 种蝽象 **EST** 同工酶聚类图

2.4 Locus and allele of EST isozymes

All 4 loci resolved for *S. minutus* were polymorphic except for locus 3. The number of alleles per locus ranged from one to six, with a mean of 4.25. Within populations of *R. pedestris*, 4 of the 5 loci were polymorphic on average. The number of alleles per polymorphic locus within populations ranged from 1.80 (Mount Cuihua) to 3 (Xunyangba), with a mean of 2.33. Genetic diversity within the species was relatively high. The results are listed in Table 2 in detail (Date about locus and allele of EST isozymes of *A. bicolor* and *A. notatus* were absent for poor amount of captured insects).

Table 2 The number of alleles of the esterase isozymes

表 2 **EST** 同工酶等位基因数

Species	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Total allele	Average locus allele
S. minutus	5	6	1	5		17	4.25
R. pedestris							
population of Louguantai	3	2	2	1	3	11	2.20
population of Mount Cuihua	2	2	2	1	2	9	1.80
population of Xunyangba	3	4	3	1	4	15	3.00

3 Discussion

3.1 Enzymegraph about the four stink bugs

The esterase isozyme enzymegraph about the four Coreidae bugs could be studied on three levels.

3.1.1 Intraspecific analysis The features of EST isozyme were relatively intraspecific stable as all Coreid bugs examined so far. All the four species, such as *S. minutus* and *R. pedestris*, shared most of the EST isozymes either between the sexes or among individuals (Fig. 1). This means these species are relatively intraspecific stable both hereditarily and morphologically. The clustering analysis showed that sex difference could not affect the taxonomy of species (Fig. 3). *S. minutus* and *R. pedestris* showed phenotype polymorphism. The isozyme polymorphism and genetic variability of individuals within a Coreidae species may result from the pair of different alleles at the same loci. And sex-linked gene may result in the difference between female and male within species[11]. The variances between sexes are more significant than those of the individuals and are less obvious than the differences among interspecific. The extent of the variances among genera and species is according to their morphological traits.

3.1.2 Interspecific comparisons As we know, those three Rhopalinae species can not be identified easily based on their homoplastic morphological characteristics. With reference to phylogenetic clustering based on esterase isozymes (Fig. 3), we conclude that these three species are dissimilar in heredity. By cluster analysis within Rhopalinae subfamily, we can see euclidean distance between *S. minutus* and *A. notatus* is 3.629, and 3.24 within two congeneric species in the genera *Aeschyntelus*. The analysis reveales that the bug community structure is remarkably similar within each genus, with small but significant differences between species. In contrast, considerable differences were found between the two genera *Stictopleurus* and *Aeschyntelus*. Differences in the morphological classification are also reflected in the enzyme spectrum and clustering results. From this rather limited investigation, it appears that the conclusion is in agreement with traditional and comparative morphological classification.

3.1.3 Intrapopulation comparisons From the above zymography and gene expression analyses, we can see that the three *R. pedestris* populations with small but significant differences between sampling sites, the frequency of different alleles on some sites may be the main reason for which caused different geographical differences among populations of EST isozymes. Some information indicate that a single population contains common genetic information (at least 85%～90%) existing in this species[12]. But this limited work can not come to these conclusions. For the different geographic populations of genetic differentiation, genetic distance comparison is of great significance. In addition, the polymorphic loci of the study could be planted in accordance with sub-classifica-

tion. However, no further study is carried out.

3.2 Locus analysis and allele variation of the EST isozymes

The loci and alleles of the EST isozymes of those two Coreid bugs with large samples were presumed in this article (Table 2). The genetic analysis showed that the esterase isozymes of each species were controlled by 4 or 5 polymorphic loci. Genetic polymorphism of EST isozyme exists in Alydinae and Rhopaline bugs involved in, and different genotypes result in isozyme polymorphism within a species. On the other hand, Homozygote represented one band in the gel and heterozygote 2 bands with the same concentration and colouration[9]. Esterase isozymes possessing such representative phynotype were observed, indicating that all the esterase presented as monomeric enzyme toward naphthyl ester substrates except for those which were dominated by monomorphic loci of the four Coreidae species.

Nevertheless, the phylogenetic reconstruction proposed here should be corroborated by further studies involving both larger samples and molecular biology evidence.

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