

SIGNIFICANCE OF PASSIVE MIGRATION IN EVOLUTION OF INSECTICIDE RESISTANCE IN THE MOSQUITO *CULEX PIPPIENS* (DIPTERA: CULICIDAE)

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Abstract In order to determine the combined effects of migration and gene flow on evolution of insecticide resistance in the mosquito *Culex pipiens*, four samples were collected in China, among which two were collected along the railway from Beijing to Guangzhou. Bioassay data showed that the resistance levels of the four populations to dichlorvos were high and to parathion moderate as compared with the susceptible strain and there was no significant difference among the four populations to the same organophosphate (OP) insecticide. Starch electrophoresis was done to identify the frequency of known overproduced esterases and to analyze genetic diversity among various populations by electrophoretic polymorphism of five presumably neutral loci. The results indicated that the gene flow between populations existed and the number of effective migrants (N_m) was related to collection geography (N_m from 1.67 to 40.07). In contrast with lower genetic differentiation between two nearby populations (between GZ and ZS, ZZ and SQ) and higher genetic differentiation between two distant populations (between GZ and ZZ), there was a significant and inconsistent difference in the distribution of resistance alleles, A2-B2 when explained only with active migration. This divergent situation could be straightened out when considering passive migration (such as railway transport) which increased the spread of A2-B2 along the railway, i. e., in GZ and ZZ. The resistance alleles, A2-B2, dispersing to around areas by active migration suffered from the limitation of gene flow and the speed of invasion.

Key words *Culex pipiens*, gene flow, passive migration, genetic diversity

1 INTRODUCTION

In the past forty years, the wide use of organophosphate (OP) insecticides to control agricultural and public health pests has been a powerful agent of selection in field population of many insect species that have developed various degrees of resistance.

The super-locus Ester is one of two genome areas in *Culex pipiens* involved in organophosphate (OP) insecticide resistance (Lenormand *et al.* 1998). This super-locus is composed of two loci on chromosome 2, Est-3 and Est-2, separated by an intergenic DNA fragment of 2–6 kb, and both loci encode for detoxifying esterases which degrade or sequester OPs before they reach their target (Rook-

er *et al.* 1996). This over-production is the result of two nonexclusive mechanisms: gene amplification of one or both loci, or gene regulation (Raymond *et al.* 1998). In 1995, we started to do some researches in the mechanism of resistance to OPs and the distribution of different resistance alleles in China (Qiao *et al.* 1995, 1998). In *Culex pipiens*, four Ester alleles involved in resistance have been described clearly in China (Liu *et al.* 2001, Qiao *et al.* 1998, Weill *et al.* 2001): three correspond to the coamplification of both Est-2 and Est-3 loci (Ester2, Ester8, Ester9, encoding esterases A2-B2, A8-B8, A9-B9, respectively), one corresponds to the exclusive amplification of Est-2 (EsterB1, encoding esterase B1). The results from two

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methods, starch gel electrophoresis and RFLP, are consistent (Liu *et al.* 2001, Qiao *et al.* 1998, Berticat *et al.* 2000). It should be noted that these four types of esterases can be discriminated in starch gel electrophoresis under our experiment conditions because they have distinct electrophoretic mobility (Weill *et al.* 2001).

The dynamics of insecticide resistance alleles in field populations is dependent on the classical evolutionary factors: mutation, migration, drift and selection. For the mosquito *Culex pipiens*, it has been shown that mutation is a limiting factor (Raymond *et al.* 1991). In contrast, the role of migration in the evolution of resistance alleles seems important, as illustrated by some resistance alleles which have a unique origin and now have a wide distribution (Raymond *et al.* 1991, Qiao *et al.* 1995). Resistance alleles are obviously advantageous in treated areas so that their frequencies increase with selection. However, in non-treated areas or when control is scarce, resistance alleles are at a disadvantage relative to susceptible ones (Chevillon *et al.* 1995). Therefore, the balances between positive and negative selection pressure, migration and drift, mainly determine the local dynamics of resistance alleles.

We chose four sites to collect field *Culex pipiens* populations where OP insecticides had been widely used: two were along the railway, and the other two were near the above two sites respectively. Through the distribution of resistance alleles and differences among field populations, we analyzed the role of migration (active migration and passive migration) and gene flow in the dispersal of resistance alleles in *Culex pipiens*.

2 MATERIALS AND METHODS

2.1 Mosquito samples

Four breeding sites were sampled as egg rafts, larvae and pupae from June to August 2001 (Table 1). GZ and ZZ were collected along the railway from Beijing to Guangzhou. SQ was near ZZ and ZS near GZ. Parts of the larvae were immediately used for bioassay. The rest were reared in the laboratory

until adult stage, and then deeply frozen for further analyses. Every field population was about several thousands of larvae.

Table 1 Collection locations of the *Culex pipiens* complex populations.

Population	Code	location	Time (year /month)
GUANGZHOU	GZ	N23. 13 °, E113. 29 °	2001/5
ZHONGSHAN	ZS	N22. 50 °, E113. 41 °	2001/5
ZHENGZHOU	ZZ	N34. 74 °, E113. 65 °	2001/8
SHANGQIU	SQ	N34. 48 °, E115. 58 °	2001/8

2.2 Bioassay

Bioassays were performed on 4th instar larvae with dichlorvos and parathion as described by Raymond *et al.* (1987). Beijing susceptible strain (Beijing-s), provided by the Institute of Microbiology Epidemiology, Academy of Military Medical Sciences, untreated by any insecticide for several decade generations, was used as control. Mortality data were analyzed with PROBIT software (version 3.3) (Ratsira *et al.* 1993).

2.3 Electrophoresis

Presence or absence of highly active esterases was determined on single mosquito homogenates by starch gel electrophoresis in TME 7.4 buffer system (Tris 0.1 mol/L, Malic anhydride 0.1 mol/L, EDTA 0.01 mol/L, MgCl₂ · 2H₂O 0.01 mol/L) according to Pasteur *et al.* (1988). In each gel, mosquitoes from strains with known overproduced esterases were run as control: strain SELAX with esterases A2-B2, TEM-R with B1, MAO with A8-B8.

The electrophoretic polymorphism of five enzymatic loci was studied with starch gel electrophoresis under TME 7.4 buffer system as described by Pasteur *et al.* (1988): ME (Malic enzyme, E. C. 1.1.1.40), MDH-1, MDH-2 and MDH-3 (Malate dehydrogenase, E. C. 1.1.1.37), GPD (Glycerol-3-phosphate dehydrogenase, E. C. 1.1.1.8). Strains used for electrophoretic mobility reference were the same as above.

2.4 Statistics

F-statistics was calculated according to Weir and Cockerham (1984) by the GENEPOP package (version 3.1b). The number of effective migrants (Nm) was estimated by one method from F-statistics according to the equation

$$Nm = (1/F_{ST} - 1) / 4 \quad (\text{Wright 1969}).$$

3 RESULTS

3.1 Larval Bioassay

Resistance of the four populations to two kinds

of OP insecticides, dichlorvos and parathion, was studied by bioassay, using Beijing susceptible strain as control (Table 2). They all displayed a high resistance to dichlorvos (RR from 29 to 21), a moderate but significant resistance to parathion (RR from 11 to 7). There was no significant difference among the four populations in the two OP insecticide resistance, which indicated, at least in part, the selection caused by OP insecticides was similar among the four field populations.

Table 2 Insecticide-resistant levels of the four populations of *Culex pipiens* complex.

Insecticide	Code	N	Slope (±SE)	LC ₅₀ (mg/L)	RR
Dichlorvos	Beijing-s	75	4.91 ±0.194	101.833	1.000
	GZ	83	1.68 ±0.133	2980.306	29.267
	ZS	96	2.29 ±0.033	2181.929	21.427
	ZZ	78	3.29 ±0.248	2684.211	26.359
	SQ	91	2.90 ±0.200	2369.921	23.273
Parathion	Beijing-s	79	1.45 ±0.077	0.345	1.000
	GZ	75	0.13 ±0.008	3.744	10.852
	ZS	75	0.13 ±0.008	4.004	11.606
	ZZ	87	0.19 ±0.015	2.874	8.330
	SQ	77	0.17 ±0.010	2.621	7.597

3.2 Overproduced esterases

Electrophoretic studies revealed the presence of overproduced esterases B1, A2-B2, A8-B8, A9-B9, but their frequencies and activity were different from each other (Figure 1 and Table 3).

Highly active B1 presented in ZZ and SQ, but the frequency of B1 was distinct between them. Moderately active B1 occurred in GZ and ZS also with different frequency.

The associated esterases A8-B8 were observed in all samples at high frequencies and their activity is higher in ZZ and SQ than in GZ and ZS. The associated esterases A9-B9 appeared in ZZ and SQ at low but significant frequencies and in GZ and ZS at high frequencies. The associated esterases A2-B2 were found in ZZ and GZ at similar and high frequencies, but not observed in ZS and SQ.

Table 3 The frequencies of over-produced non-specific esterases among the four populations.

Populations	Sample size	Esterase B1	Esterases A2-B2	Esterases A8-B8	Esterases A9-B9
GZ*	107	21.36 %	34.95 %	53.40 %	63.11 %
ZS	88	4.45 %	0	53.40 %	47.47 %
ZZ	94	48.94 %	46.81 %	48.94 %	5.32 %
SQ	50	20.00 %	0	66.00 %	16.00 %

* sited from Zhang *et al.* 2003.

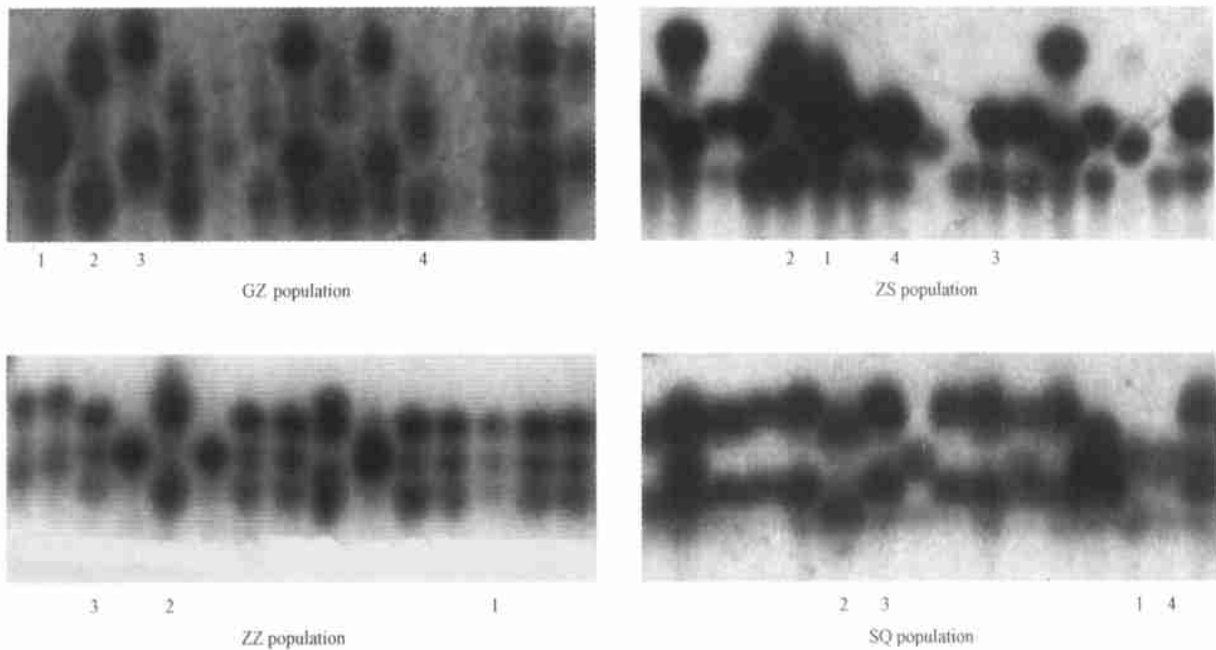


Fig. 3 Electrophoresis patterns of over-produced esterases in *Culex pipiens* populations. No. 1 B1 (TEM-R) ; No. 2 A2-B2 (SELAX) ; No. 3 A8-B8 (MAO) ; No. 4 A9-B9.

3.3 Insecticide non-selected genes

Among the 5 presumably neutral enzymatic loci investigated, all presented polymorphism and were used to analyze the population differentiation. Importance of genetic exchange between populations was estimated by computing the F_{st} parameter according to Weir and Cockerham, deriving from it the number of effective migrants (N_m) (Table 4). F_{st} ranged from 0.1307 to 0.0062 (N_m from 1.67 to 40.07). These results indicated that the differentiation among populations existed significantly and the gene flow was limited by geography. The highest N_m existed between GZ and ZS ($N_m = 40.07$), the second highest N_m between ZZ and SQ ($N_m = 12.51$).

Table 4 Matrix of the F_{st} values when testing genetic differentiation (below diagonal) and the number of effective migrants (N_m) (above diagonal) coefficients among populations.

Populations	GZ	ZS	ZZ	SQ
GZ	—	40.07	4.74	2.81
ZS	0.0062	—	2.21	1.67
ZZ	0.0501	0.1017	—	12.51
SQ	0.0816	0.1307	0.0196	—

3.4 Comparison between esterase distribution and genetic differentiation

The genetic differentiation was low between two nearby populations, GZ and ZS, ZZ and SQ, respectively, but the distribution of overproduced esterases A2-B2 was inconsistent with it. The esterases A2-B2 existed in GZ and ZZ at high frequencies, but in ZS and SQ, no A2-B2 were discovered. This divergent situation could not be explained reasonably only with flight migration (active migration) because the bioassays showed no significant difference correlated with the intensity of insecticide uses to various populations, and in flight migration the distribution of selected genes (resistant alleles) and non-selected genes (neutral genes) should be similar. This conflict could be explained if the passive migration (such as railway transport) was taken into account to increase the intensity of genetic exchange between GZ and ZZ. The flight migration was the main pattern of gene flow between ZZ and SQ or GZ and ZS.

It is probable that several generations are needed for a neutral gene from one location to another. For a selected gene, the number of generations is certainly shorter if OP insecticide treatment

is widespread, because this treatment will rapidly increase the resistant allele frequency in each new site, and such increases its chance to become a future colonizing population.

4 DISCUSSION

Mosquito *Culex pipiens* populations have developed resistance to OP insecticides (dichlorvos and parathion) in the most areas of China. Four non-specific esterase alleles (encoding for esterases B1, A2-B2, A8-B8, A9-B9, respectively) known conferring resistance to OPs were described clearly. Overall, esterase frequencies and resistance ratio are correlated positively with the intensity of insecticide application.

The heterogeneous distribution of A2-B2 within the 4 populations could indicate a recent introduction or a heterogeneous selection. The bioassays did not show significant differentiation to the same insecticide among these populations. In 1995, A2-B2 were observed in GZ at a lower frequency than the present data and not observed in Wuhan which was localized between ZZ and GZ and along the railway from Beijing to Guangzhou (Sun *et al.* 2000). The heterogeneous distribution of A2-B2 is probably transitory, as extensive gene flow at this geographical scale will bring them into all treated areas rapidly. The equilibrium frequencies depend on the value of their fitness cost. This also fits for A8-B8 and B1.

Genetic differentiation (measured by *F_{st}*) between populations significantly increasing with their geographic position was in agreement with isolation by distance. However, there was also a significant decrease in differentiation between populations when traffic increased, indicating the railway could play an important role in mosquito passive migration. In addition, there were direct and indirect evidences of large-scale migration of this mosquito by passive transportation (Chevillon *et al.* 1995, Pasteur *et al.* 1995), and the presence of females with A2-B2 in southern France had been documented (Rivet *et al.* 1993).

It is unlikely that the present situation will

provide a strong and opposite selection pressure to adjacent areas so as to lead to a speciation event, due to the high gene flow between these populations. It is perhaps a general conclusion for insect resistance that mosquitoes display a big propensity to maintain a high gene flow level with extremely large populations. Clearly, migration can not be ignored as a driving force in studying and monitoring the insecticide-treated populations of the *Culex pipiens*.

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被动迁移在抗性进化中的作用

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为了明确迁移和基因交流在杀虫剂抗性基因进化中的作用, 我们从四个不同的地区采集有机磷抗性的库蚊野生种群, 利用淀粉电泳鉴定了各种群中存在的已知过量产生酯酶的分布频率, 并通过 5 个假定的中性位点的电泳多态性分析了种群间的遗传多样性。结果表明种群间的基因交流是存在的, 遗传分化与地理位置存在一定关系, 而抗性等位基因 A2-B2 的分布却与种群间的遗传分化不一致。对这种差异的解释是: 被动迁移(铁路运输等)加速了抗性基因的交流, 而当抗性基因以自然迁飞的方式向周围地区扩散时, 却是一个相对缓慢的过程。

关键词 库蚊 基因交流 被动迁移 遗传多样性

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