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Diversity and frequencies of genetic mutations involved in insecticide resistance in field populations of the house fly (*Musca domestica* L.) from China

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ABSTRACT

Insecticides have been extensively used for house fly control in China, with dichlorvos and deltamethrin being widely used. Knowledge about the current status of insecticide resistance and the underlying genetic changes is crucial for developing effective fly control strategies. The susceptibility to dichlorvos and deltamethrin, and the frequencies of genetic mutations involved in insecticide resistance were studied in five field populations of the house fly collected across China. Bioassay results show that flies exhibit 14- to 28-fold resistance to dichlorvos and 41- to 94-fold resistance to deltamethrin, indicating that dichlorvos and deltamethrin resistance are common in house fly populations in China. Molecular analysis reveals that flies from the five various locations carry resistance alleles at multiple loci and have diverse allelic types, different relative frequencies and combinations of each allele. Four non-synonymous single nucleotide polymorphisms (SNPs) (i.e. V260L, G342A/V, F407Y) in acetylcholinesterase (*Ace*) and two mutations (W251L/S) in a carboxylesterase (*MdαE7*) were commonly present in the field house flies. The L1014H rather than L1014F mutation in the voltage sensitive sodium channel gene (*Vssc*) was widely distributed in Chinese house flies. *CYP6D1v1*, which confers pyrethroid resistance, was found in all the five tested populations in China, although its frequency in house fly from Shandong province was very low. Our results suggest that resistance monitoring and management of house flies should be customized for a given location.

1. Introduction

The house fly (*Musca domestica*) is a cosmopolitan species and important sanitary pest of humans and animals. It is a mechanical carrier of more than 100 human and animal pathogens [1], including those resistant to antibiotics [2]. In addition, house flies are one of the most serious pests at dairy, horse, sheep, and poultry facilities [3]. House fly activity results in lowered levels of egg and milk production and reduced feed conversion [3]. The control of house flies often depends on insecticides. Organophosphates (OP) and pyrethroids have been used widely as insecticides for house fly control in many countries, including China [4,5], and they continue to be the most frequently used insecticides for house fly control in China.

Extensive use of insecticides has led to resistance in house flies worldwide [6,7]. Resistance is usually associated with a few genes and a limited number of mutations in each gene. One major type of resistance to OPs is target site (acetylcholinesterase, AChE) insensitivity [8]. Six AChE mutations (V260L, A316S, G342A/V, F407Y, and G445A, numbering based on the sequence of aabys AChE, accession number AF281161, corresponding to positions 180, 236, 262, 327 and 365, respectively, if numbering is based on the mature AChE protein sequence [9]) alone or in various combinations play a role in conferring resistance [8–11]. The other major type of resistance (metabolic resistance) to OP insecticides is due to insecticide detoxification by P450 monoxygenases or carboxylesterases. OP resistance in certain strains of *M. domestica* (such as the Rutgers Diazinon-R) is associated with reduction in the carboxyesterase activity of a particular esterase enzyme [12]. Resistance to some organophosphates has been associated with lower levels of carboxylesterase in the resistant strains, and this was named the "mutant aliesterase" mechanism of resistance [13]. In the blow fly, *Lucilia cuprina*, a mutation in a carboxylesterase (*αE7*) was found to lower the activity of general esterase and increase the hydrolysis of the OP insecticide diazinon [14]. The orthologous gene has been identified in house fly and mutations in this gene may be responsible for the "mutant aliesterase" resistance to some OPs in house flies [12].

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Two major forms of pyrethroid resistance in house flies have been observed: target-site (voltage sensitive sodium channel) insensitivity and increased detoxification mediated by P450 dependent monooxygenases [15]. The molecular basis of target insensitivity is well-characterized [16]. Cloning of the full-length voltage sensitive sodium channel gene (*Vssc*) cDNA identified *kdr* (L1014F) [17], *kdr-his* (L1014H) [18] and *super-kdr* (M918T + L1014F) mutations [17]. Over-expression of *CYP6D1* confers pyrethroid resistance in the LPR strain, as well as several populations of house flies in the USA [4,19,20] and China [7].

In a previous study [7], combinations of resistance alleles for *CYP6D1* (*CYP6D1v1*) and *Vssc* (*kdr* and *super-kdr*) were detected to be associated with deltamethrin resistance in a strain of house fly (BJD) collected in Beijing, China and selected with deltamethrin. Although there are many field surveys about the status of insecticide resistance in house flies in China to various types of insecticides [21,22], little is known of the underlying genetic changes that confer resistance in field populations. In this study, we conducted a survey of resistance to the commonly recommended organophosphate (dichlorvos) and pyrethroid (deltamethrin), and investigated the genetic mutations involved in insecticide resistance in Chinese field populations of *M. domestica* across a broad geographical area.

2. Materials and methods

2.1. House flies

House flies were collected from a pig farm or municipal dumps at five different provinces (Table 1) in China during September and October in 2009. Adults (>100 individuals, designated as the parental generation) were caught by sweep net. Collections from each site were transported to the laboratory and allowed to randomly mate in order to establish corresponding laboratory colonies. Field-collected flies were stored in 100% ethanol after laying eggs and kept in −20 °C for molecular analysis. The F2 or F3 progeny flies were used for toxicity bioassays. An insecticide susceptible strain (S-lab) [23], which has been maintained in our lab without exposure to any insecticide, was used as a control strain. Flies were maintained at 25 °C and photoperiod of 12:12 h (L:D). Larvae were reared on wheat bran-based media. Adults were fed powered milk: sugar (1:1) and water *ad libitum*. According to personal communications with the employees at these sampling sites, OPs such as dichlorvos, and pyrethroids such as deltamethrin have been used in fly control.

2.2. Bioassays

Deltamethrin (99%, Roussel UCLAF) or dichlorvos (DDVP, 86.7%, Shandong Dacheng Pesticide Limited Company, China) were dissolved in acetone and applied in 0.928 μL to the thoracic notum of female house flies. Each experiment was replicated at least three times. Each bioassay consisted of twenty-five 3–5 days old house flies per dose and five or six doses that gave >0% and <100% mortality. Control groups received acetone alone. The treated insects were put in 200 mL cups covered with cheese cloth and water and food was provided. Cups with treated insects were held at 25 °C. Mortality was recorded 24 h after insecticide application. Bioassay data were pooled and analyzed based on standard probit analysis using the POLO program, after Abbott's correction for control mortality.

2.3. Isolation of genomic DNA and analysis of alleles

Genomic DNA was isolated from the adult flies which had been stored in 100% ethanol and frozen at −20 °C by the method of Rinkevich et al. [4]. Abdomens were discarded prior to the isolation of DNA.

The *Ace* fragment (~800 bp) was amplified by PCR according to Kozaki et al. [8] The PCR products of *Ace* were directly sequenced with S90mdace [8] by BGI Company (Beijing, China).

A region (~750 bp) of the *MdαE7* gene was amplified by PCR in a 20 μL reaction containing 1U Taq polymerase (Takara LA, Takara), 4 pmol of primers (Md_aliest_F and Md_aliest_R [24],) and 2 μL of genomic DNA as a template. The reaction were performed at 95 °C for 3 min, followed by 35 cycles of PCR (95 °C for 30 s, 50 °C, 30 s, 72 °C 1 min) and then extension at 72 °C for 5 min. Pilot experiments showed noisy signals in electropherogram when the PCR products were sequenced directly. Therefore, five individual flies were randomly selected for sequence analysis through TA cloning. The PCR products were ligated into pGEM and sequenced. Three to five clones were sequenced for each individual.

A fragment of *Vssc* (~350 bp) was amplified by PCR using primers of kdr-Diglong F and kdr-Diglong R, and PCR products were directly sequenced with kdr-Diglong F according to the method of Rinkevich et al. [4].

CYP6D1 5′ flanking fragments (~730 bp) were generated by PCR using primers S35 and AS2. Genotypes were determined using a PCR-RFLP method [4].

3. Results and discussion

3.1. Organophosphate and pyrethroid resistance

Insecticide resistance in the house fly has been reported by a number of surveys worldwide [23,25–30]. The susceptibility of house flies in China to two commonly recommended insecticides was determined in this study. LD_{50} values (Table 2) revealed that all five field populations collected from various locations of China differed significantly from the laboratory susceptible strain (S-Lab), showing that field populations of house flies developed resistance to both dichlorvos (14- to 28-fold) and deltamethrin (41- to 94-fold) (Table 2). In China, OPs (commonly DDVP and malathion, both are dimethyl OPs) and pyrethroids (commonly deltamethrin) have been used extensively for public health for more than 20 years (OPs were first introduced in 1952, and pyrethroids by the end of 1970's). Thus, it is not surprising that widespread resistance was found in this study. While it is uncertain whether or not

Table 1
Sampling sites of field collected house flies in 2009.

Populations	Guangdong (GD)	Shanghai (SH)	Shandong (SD)	Beijing (BJ)	Jilin (JL)
Location	Guangzhou, Guangdong Province	Feng Xian, Shanghai Municipality	Jinan, Shandong Province	Chaoyang, Beijing Municipality	Changchun, Jilin Province
Longitude	113°18′E	121°26′E	117°59′E	116°22′E	125°16′E
Latitude	23°06′N	30°54′N	36°39′N	39°59′N	43°53′N
Type of breeding sites	Dump	Pig farm	Dump	Dump	Dump