ABSTRACT  A field survey was conducted in the summer of 2003 and 2004 to evaluate resistance to deltamethrin on house flies, Musca domestica, from 17 urban garbage dumps in the cities of Beijing, Tianjin, and Zhangjiakou. Bioassays were carried out by topical application with technical deltamethrin to measure the levels of resistance to deltamethrin. Tests for synergism with piperonyl butoxide (PBO) were used to study the occurrence of metabolic resistance mechanisms. Competitive polymerase chain reaction (PCR) amplification of specific allele (cPASA) assay was used on individual house flies from these field populations to detect the presence of the kdr and super-kdr alleles associated with pyrethroid resistance. Another PCR followed by labeled allele-specific oligonucleotide hybridization (PCR-ASO) confirmed the presence of the mutation and hence checked the accuracy of cPASA. Deviation of genotype ratios from Hardy-Weinberg equilibrium was tested. Relative to a susceptible laboratory strain, field house flies from all populations exhibited a 13- to 250-fold greater resistance to deltamethrin in all the populations of house flies at the LD50 and the PBO synergism coefficient was 26- to 124-fold greater in 11 populations collected from Beijing. The super-kdr allele was not found in any of the populations. Results of PCR assays showed that the kdr allele was present at various frequencies in the field populations. ASO-PCR showed that the cPASA method had given only 1 of 33 of false positives. Deviation of frequencies of kdr alleles genotypes from the Hardy-Weinberg ratios was found in the BJF-1, BJF-2, BJF-3, BJHD, and BJXC populations. Regression analysis indicated a significant correlation between kdr allele frequencies and the levels of resistance to knockdown resistance by deltamethrin. The results validate the role of the PCR-based molecular assay as a diagnostic tool in monitoring resistance to pyrethroids and also to provide useful information on population genetics of house fly resistance to pyrethroids.

KEY WORDS  pyrethroid resistance, kdr, Musca domestica, deltamethrin

THE HOUSE FLY, Musca domestica, is the probable mechanical vector of >65 human and animal intestinal diseases (Greenberg 1965), and control of house flies with deltamethrin space spraying has been shown to significantly reduce incidence of diarrheal diseases in Pakistan (Chavasse et al. 1999) and Gambia (Emerson et al. 1999). Recently, enterohemorrhagic Escherichia coli O157:H7 was isolated from house flies in Japan (Iwasa et al. 1999). Four kinds of pest control programs promoted by the central and local offices of National Patriotic Health Movement (NPHM) are carried out in many cities in China. Aerosols or space sprays with pyrethrum synergized with piperonyl butoxide or organophosphorous (OP) formulated with pyrethroid are commonly used for house fly control indoors and outdoors around dwelling houses, as well as dustbins or garbage dumps, but these give only temporary control. It was found that extensive use of pyrethroid insecticides for house fly control has led to widespread pyrethroid resistance (Sun and Fan 1993, Huo and Wang 1997, Lin et al. 1999, Liu and Dong 2001), and this has become a global problem (Keiding 1999).

Knockdown resistance (kdr) was first identified in M. domestica ~60 yr ago (Milani 1954). A stronger form of kdr known as super-kdr was identified by Sawicki (1978). Unlike resistance caused by enhanced metabolic detoxification, knockdown resistance is unaffected by synergists that inhibit insect esterases and mono-oxygenases. Knockdown resistance is caused by a reduction in the sensitivity of the insect nervous system to pyrethroids. It has been proved that knockdown resistance is associated with several point mutations in voltage-sensitive sodium channel genes. Through comparison of partial and complete sequences from 15 susceptible, kdr, and super-kdr house fly strains, two point mutations (L1014 F and M918T) have been shown to be associated with knockdown resistance (Ingles et al. 1996, Miyazaki et al. 1996, Williamson et al. 1996). Mutation of leucine to phenylalanine at amino acid residue 1014 (designated L1014 F) occurs in all kdr and super-kdr strains, whereas mutation of methionine to threonine at residue...
and due 918 (designated M918T) appears only in super-
kdr strains (Ingles et al. 1996, Miyazaki et al. 1996, Williamson et al. 1996). Up to now, the L1014 F mutation have also been reported in seven other pest species: Blattella germanica (Miyazaki et al. 1996, Dong 1997), Hematobia irritans (Guerrero et al. 1997), Anopheles gambiae (Martinez-Torres et al. 1998), Platulla xylostella (Schuler et al. 1998), Leptinotarsa decemlineata (Lee et al. 1999), Myzus persicae (Mar-
tinez-Torres et al. 1999a), and Culex pipiens (Mar-
tinez-Torres et al. 1999b). However, more direct ev-

evidence that the kdr mutation is responsible for knockdown resistance has been reported recently. Heterologous expression of kdr α subunits with the L to F change in the IIS6 region reduced channel sen-
sitivity to pyrethroid insecticides compared with the channels in wildtype insects (Smith et al. 1997, Vais et al. 1997, Schuler et al. 1998, Soderlund and Knipple 1999, Tan et al. 2002).

Because of continual increase of knockdown resis-
tance, the monitoring of knockdown resistance level in urban house fly populations has become a crucial problem, which provides useful information for choosing different kinds of insecticides and determining the dosage applied in pest management. Traditionally, the resistance level is evaluated by bioassay method, which is important in measuring the levels of resis-
tance and cross-resistance in pest populations. How-
ever, bioassay data give no information on the geno-
typic composition or the frequency of resistance genes in a population. Recently, as more understanding of the molecular mechanism of insecticides resistance has been acquired, molecular monitoring of resistance level has become feasible. To validate identification of the point mutations responsible for insecticide resis-
tance, single-strand conformation polymorphism (SSCP), mini-sequencing, polymerase chain reaction (PCR) amplification of specific allele (PASA), and multiplex PCRs have been carried out (Guerrero et al. 1998, Zhang et al. 1999, Zhu et al. 2000, Valles et al. 2003). The primary objective of this study was to monitor the current knockdown resistance of the house fly in some areas in Northern China, to test for house fly susceptibility to deltamethrin and identify whether the L1014 F or M918T nucleotide substitu-
tions are present, and to investigate the distribution of kdr-allele frequencies in urban populations. PASA was developed to identify kdr genotypes in wild popu-
lations and to investigate if there was a correlation between the kdr allelic frequency and the level of target site resistance to deltamethrin. It has been re-
ported that, with the PASA technique, there is a high rate of false positivity, so a PCR-allele specific oligo-
nucleotide probe (PCR-ASO) was further applied to validate the PASA results. This was done by PCR followed by sequence-specific oligonucleotide probing for detection of the kdr gene. Finally the deviation of kdr allelic frequency from the Hardy-Weinberg ratio was tested in all the populations of house flies.

**Materials and Methods**

**Insect Strains.** Field house flies were collected by using sweep nets around the dustbins and garbage dumps in the summers of 2003 and 2004. The populations, named TJNK, TJJG, TJDL, TJXQ, and TJNJ, were collected randomly from different districts in Tianjin city. BJZZ, BJDD, BJF-1, BJF-2, BJF-3, BJHD, BJXC, BJXWJ, BJLLT, BJWLJ, and BJBS populations were collected from Beijing city, and the ZJK population was collected from Zhangjiakou city (Table 1). All field populations were routinely reared as de-
scribed by Scott et al. (2000). The adult flies for genomic DNA extraction were frozen using liquid nitrogen and stored in 95% ethanol at −20°C.

**Bioassay, Technical grade deltamethrin (95%, Rou-
sel-Uclaf, Paris, France) insecticide was tested, and bioassays were carried out by topical application of a 1-μl drop of insecticide in acetone solution to the thoracic notum of 3- to 5-d-old female flies. Each of the three replicates consisted of 15–25 flies/dose and at least three to five doses, giving >0 and <100% kill. For tests of synergism, piperonyl butoxide (PBO) was ap-
plicated at a dose of 10 μg/fly in a 0.5-μl acetone solution to the thoracic notum 1 h before dosing with deltameth-

Flys were bioassayed beginning with the first or second generation of adults produced by the field-
collected flies. One susceptible (Lab) house fly strain that has not been in contact with insecticides for around 20 yr and has been reared routinely in the laboratory served as the reference for molecular di-
agnostic tests. All tests were carried out at 26 ± 1°C and were replicated three times. Mortality was as-
essed after 24 h holding at 26 ± 1°C, and flies were fed 0.1% glucose water tampons. Bioassay data were pooled and analyzed with log dose-probit mortality software (Finney 1971, Raymond et al. 1993). Resis-
tance ratios were calculated by dividing the LD₅₀ values for different field populations by the LD₅₀ of the susceptible Lab strain.

**Genomic DNA Isolation.** Genomic DNA was iso-
lated from 39 to 50 individual adult house flies per sample of 17 field populations and 1 Lab strain with the method of Williamson et al. (1993). The whole individual house fly was homogenized in 300 μl extraction buffer (0.1 M Tris-HCl, pH 8.8, 0.1 M ethylenediamine tetra-acetic acid, 1% sodium dodecyl sulphate). The homogenate was incubated at 56°C overnight after adding 50 μg protease K. Then, 70 μl of 5 M potassium acetate was added, incubated on ice for 2 h, and centrifuged at 12,000 rpm for 20 min. The supernatant was extracted with phenol, and DNA was precipitated with ethanol. House flies yielded 10–15 μg DNA.

**Assay for kdr and Super-kdr Genotype.** For the determination of kdr genotype, the method of competitive PASA (cPASA) was adapted from the work of Jamroz et al. (1998), Martinez-Torres et al. (1999b), and Zhang et al. (1999), with some modification. A test using two PCR reactions for each individual was de-
veloped to diagnose the kdr allele. The two reactions were exactly the same except that one contained a
Table 1. LD<sub>50</sub> of dose-mortality regression of house flies tested with deltamethrin and deltamethrin + PBO

| Population | Date tested | Number | Deltamethrin | Deltamethrin + PBO | Synergism coefficient* | RR† |
|------------|-------------|--------|--------------|---------------------|------------------------|-----|
|            |             | LD<sub>50</sub> (95% CL) (µg/µl) | LD<sub>50</sub> (95% CL) (µg/µl) | Slope (SE) | LD<sub>50</sub> (95% CL) (µg/µl) | LD<sub>95</sub> (95% CL) (µg/µl) | Slope (SE) | |
| TJNK       | Summer 2003 | 230    | 0.0640 (0.0357-0.110) | 11.829 (2.348-59.581) | 0.73 (0.25) | — | — | 67.44 |
| TJXQ       | Summer 2003 | 230    | 0.0124 (0.00630-0.0247) | 6.480 (1.078-38.957) | 0.61 (0.15) | — | — | 13.07 |
| TJJN       | Summer 2003 | 230    | 0.0353 (0.0325-0.0632) | 0.726 (0.386-1.365) | 1.37 (0.15) | — | — | 47.73 |
| TJTG       | Summer 2003 | 230    | 0.0143 (0.0082-0.0231) | 1.209 (0.364-4.921) | 0.55 (0.18) | — | — | 15.07 |
| TJDL       | Summer 2003 | 230    | 0.0058 (0.00373-0.00694) | 0.597 (0.340-1.048) | 1.54 (0.53) | — | — | 53.53 |
| BJZZ       | Summer 2003 | 375    | 0.0908 (0.00667-0.0950) | 0.678 (0.438-1.049) | 1.78 (0.25) | — | — | 85.14 |
| BJDD       | Summer 2003 | 375    | 0.0756 (0.00647-0.09056) | 0.673 (0.406-1.114) | 1.76 (0.19) | — | — | 82.82 |
| ZJK        | Summer 2003 | 375    | 0.049 (0.0375-0.0640) | 1.310 (0.548-3.129) | 1.15 (0.09) | — | — | 51.63 |
| BJF-1      | Summer 2004 | 190    | 0.0503 (0.0379-0.0667) | 0.382 (0.213-0.685) | 1.57 (0.55) | 268 | 0.0018 (0.0012-0.0027) | 0.0277 (0.0162-0.0474) | 1.39 (0.14) | 27.94 |
| BJF-2      | Summer 2004 | 200    | 0.1002 (0.0770-0.1304) | 0.721 (0.432-1.202) | 2.05 (0.25) | 360 | 0.00141 (0.00070-0.00185) | 0.0233 (0.0134-0.0405) | 1.35 (0.078) | 71.06 |
| BJF-3      | Summer 2004 | 188    | 0.1305 (0.1056-0.1584) | 1.172 (0.592-2.319) | 1.78 (0.31) | 166 | 0.00331 (0.00268-0.00528) | 0.0630 (0.0234-0.170) | 1.29 (0.27) | 42.15 |
| BJHD       | Summer 2004 | 288    | 0.2377 (0.2012-0.2809) | 1.194 (0.844-1.888) | 2.35 (2.95) | 235 | 0.00201 (0.00158-0.00256) | 0.0168 (0.0102-0.0279) | 1.78 (0.33) | 118.36 |
| BJXC       | Summer 2004 | 237    | 0.1567 (0.1239-0.1982) | 1.048 (0.698-1.573) | 1.99 (0.57) | 174 | 0.001 (0.000579-0.00114) | 0.0260 (0.00199-0.00340) | 3.96 (0.81) | 156.7 |
| BJWXW      | Summer 2004 | 267    | 0.1564 (0.1117-0.2079) | 2.379 (1.113-5.087) | 1.39 (0.19) | 278 | 0.00356 (0.00279-0.00534) | 0.0512 (0.0391-0.165) | 1.24 (0.25) | 40.32 |
| BJLLT      | Summer 2004 | 234    | 0.1275 (0.094-0.1724) | 1.475 (0.827-2.526) | 1.56 (0.27) | 162 | 0.00152 (0.00116-0.00199) | 0.0097 (0.00566-0.0146) | 2.12 (0.63) | 83.88 |
| BJLWJ      | Summer 2004 | 251    | 0.1039 (0.0775-0.139) | 0.904 (0.551-1.407) | 1.75 (0.28) | 144 | 0.00392 (0.00240-0.00640) | 0.0319 (0.0158-0.0642) | 1.81 (0.63) | 36.51 |
| BJRSS      | Summer 2004 | 224    | 0.1309 (0.1125-0.2023) | 1.615 (0.931-2.800) | 1.60 (0.27) | 208 | 0.00122 (0.000649-0.00230) | 0.0703 (0.0237-0.208) | 0.93 (0.19) | 123.09 |
| Lab        | Summer 2004 | 139    | 0.000949 (0.000602-0.00150) | 0.0230 (0.00648-0.0361) | 1.16 (0.17) | 138 | 0.000919 (0.000604-0.00140) | 0.01839 (0.00556-0.0597) | 1.26 (0.29) | 1.03 |

* Synergism coefficient equals LD<sub>50</sub> of deltamethrin test divided by LD<sub>50</sub> of deltamethrin + PBO test.
† Resistance ratio using deltamethrin alone equals test LD<sub>50</sub> divided by Lab colony LD<sub>50</sub>.
sense-specific primer ending with the base C, the susceptible codon (CTT), and the other contained a sense-specific primer ending with the base T, the kdr codon (TTT). Thus, two allele-specific inner primers were designed: sense primer A1, 5′-CCACC-GTCTGTGATGCCAATC-3′ and resistant sense primer A2, 5′-CCACC-GTCGTGATCCGAATT-3′. Two additional allele-nonspecific outer primers were based on the sequence immediately downstream from the mutation site and one sense primer far upstream. The sequence of two allele-nonspecific outer primers are as follows: sense primer (P1), 5′-CTGGAATT-TCAACGACTTC-3′ and anti-sense primer (P2), 5′-GCAAGCTAGAAAAGATTAAAG-3′.

A genomic DNA fragment of an individual of the Lab strain was amplified with P1 and P2 primers in a total reaction volume of 30 µl consisting of 80–100 ng genomic DNA, 14 mM Tris-HCl, pH 8.3, 70 mM KCl, 4.5 mM MgCl2, 0.15 mM each dNTP, and 0.67 U TakaRa rTaq. PCR conditions were one cycle of 94°C for 2 min and 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, followed by one cycle of 72°C for 5 min. After sequencing, one intron manifested its position and the size, which was conserved in the domain II region of the sodium channel gene in *M. domestica* (Martinez-Torres et al. 1998). Competitive PASA was performed in accordance with the standard procedure (Martinez-Torres et al. 1998). Competitive PASA was performed according to the standard procedures were performed according to the standard protocol of Bonferroni (Rice 1989). Conformity of genotype ratios to Hardy-Weinberg equilibrium was tested by the probability test using Genepop and was also tested assuming that the alternative hypothesis H1 was heterozygote deficiency or heterozygote excess according to Raymond and Rousset (2003).

**Statistical Analysis.** Probit analysis of dose-mortality data were conducted using POLO-PC to determine the LD50 for each bioassay and to compare the PBO- and non-PBO-treated flies (Russell et al. 1977). Resistance ratios were calculated by dividing the respective LD50 by the LD50 of the susceptible Lab strain. PBO synergism coefficients were derived by dividing the LD50 of the sample treated with deltamethrin alone by the LD50 of the sample exposed to deltamethrin in the presence of PBO.

To assess whether the kdr allelic frequency of the house fly populations can affect the pyrethroid resistance level, the relationship between allelic frequency and the LD50 for deltamethrin was analyzed with SAS Software 6.22 (SAS Institute). Coefficients of correlation were calculated between the kdr allelic frequency in different populations and the corresponding LD50 values for deltamethrin. To determine if the correlation was significant, the null hypothesis (*r* = 0, i.e., variable uncorrelated) was tested by a t-test with *n* = 2 df. The level of significance of each test was adjusted to take into account the other tests by the sequential procedure of Bonferroni (Rice 1989).

Conformity of genotype ratios to Hardy-Weinberg equilibrium was tested by the probability test using Genepop and was also tested assuming that the alternative hypothesis H1 was heterozygote deficiency or heterozygote excess according to Raymond and Rousset (2003).
Table 2. *kdr* allele frequency of house fly population monitored by ePASA

| House fly population | Sex | Total numbers of individuals | *kdr* (R) alleles Frequency | Genotype Genotype frequency |
|----------------------|-----|-----------------------------|----------------------------|-----------------------------|
|                      |     |                             | SS | SR | RR |                 | SS | SR | RR |
| TJNK*                | Both | 50                          | 20.00% | 32 | 16 | 2             | 64.00% | 32.00% | 4.00% |
| TTJC*                | Both | 50                          | 8.00%  | 42 | 8  | 0             | 54.00% | 16.00% | 0.00% |
| TJDL*                | Both | 50                          | 9.00%  | 41 | 9  | 0             | 52.00% | 18.00% | 0.00% |
| TXQ*                 | Both | 50                          | 8.00%  | 42 | 8  | 0             | 54.00% | 16.00% | 0.00% |
| TJJN*                | Both | 50                          | 11.00% | 39 | 11 | 0             | 75.00% | 22.00% | 0.00% |
| BJZZ*                | Both | 50                          | 19.00% | 33 | 15 | 2             | 66.00% | 30.00% | 4.00% |
| BJJD*                | Both | 50                          | 26.00% | 20 | 16 | 5             | 58.00% | 32.00% | 10.00% |
| ZJK*                 | Both | 50                          | 10.00% | 40 | 10 | 0             | 50.00% | 20.00% | 0.00% |
| BJF-1                | Both | 49                          | 25.30% | 24 | 24 | 0             | 49.88% | 51.02% | 0.00% |
| BJF-2                | Both | 50                          | 13.00% | 12 | 13 | 0             | 24.00% | 26.00% | 0.00% |
| BJF-3                | Both | 49                          | 66.00% | 40 | 20 | 0             | 46.00% | 32.00% | 0.00% |
| BJHD                 | Both | 39                          | 52.00% | 20 | 16 | 5             | 25.00% | 32.50% | 12.50% |
| BJXWJ                | Both | 46                          | 39.10% | 24 | 14 | 8             | 30.43% | 60.57% | 8.70%  |
| BJLKT                | Both | 47                          | 33.00% | 20 | 13 | 4             | 24.55% | 48.94% | 12.50% |
| BJWLJ                | Both | 48                          | 39.20% | 24 | 14 | 4             | 30.43% | 60.57% | 8.70%  |
| BJSS                 | Both | 47                          | 13.00% | 12 | 12 | 0             | 25.00% | 25.00% | 0.00%  |
| LAB                  | Both | 50                          | 0.00%  | 50 | 0  | 0             | 100.00%| 0    | 0     |

*No sex flies were separate in the populations.
†The result of χ² test statistics was significantly different for comparing genotype distribution in male and female house flies (χ² = 12.43, df = 2, P < 0.01).

Results
Levels of Resistance. Results of deltamethrin and deltamethrin and PBO bioassays on field populations of the house fly are given in Table 1. House flies of TJXQ and TTJC populations collected from Tianjin city had low levels of resistance to deltamethrin: 13- to 15-fold resistance was detected based on LD50. Beijing and Zhangjiakou house fly populations had much higher resistance to deltamethrin, ranging from 26- to 156-fold. Controls containing PBO or absence of PBO resulted in synergism coefficients ranging from 26- to 156-fold. Controls containing PBO (10 μg/μL) without deltamethrin showed no toxic effect on any house fly populations (data not shown).

Genotypes of House Fly Samples. Introns are known to exist in the domain II region of the sodium channel gene at conserved positions in *M. domestica* (Martinez-Torres et al. 1998). One intron is located downstream of the *kdr* mutation, and knowledge of its sequence length was necessary to discern the size of the allele by PASA. The product PCR-amplified by P1 and P2 from individual genomic DNA was sequenced and deposited in GenBank (accession no. AY309437); its size is 119 bp. After optimization, the conditions of PASA could effectively discern *kdr* homozygous and heterozygous genotypes for the L1014 F mutations in sodium channel 1. The results of PCR assays are summarized in Table 2. For the super- *kdr* genotype, based on presence or absence of the 78-bp diagnostic allele with the primers FG235, FG154, and FG155, individual flies were genotyped as homozygous susceptible (SS), homozygous resistant (RR), or heterozygous (SR). However, no mutant M918T was detected in any of the house fly samples tested, and super-*kdr* data were not listed in Table 2.

PCR assays revealed clear differences in overall *kdr* allelic frequency between resistant and susceptible house fly populations. Relatively low *kdr* allelic frequencies were detected in house flies collected from Tianjin (8–20%) and Zhangjiakou (10%), but relatively high *kdr* allelic frequencies were found in 2004 in the populations from Beijing (25–56%). The *kdr*
allele existed exclusively in heterozygous form in the Beijing house fly populations named BJF-1, BJF-2, BJF-3, BJHD, and BJXC. The detection of no resistance homozygotes among the sample sizes tested indicates highly significant deviations from Hardy-Weinberg equilibrium. In these cases homozygote frequencies were 0–84% (zero in BJF-3), and ranged from 8 to 56% susceptible homozygote frequencies were 16–88%, but one SS homozygote appeared for SR and RR (kdr homozygote). One SS homozygote individual (B-A11) identified by cPASA also appeared to have a positive signal by hybridization with resistant probe A2.

Discussion

To improve the use of existing insecticides and delay the onset of resistance and treatment failures, it is important to establish by regular surveys the real extent of insecticide resistance, even for species with an extensive resistance history. Regular surveys of resistance to insecticides of interest in relation to house fly control in China has been carried out for many years by collection of urban house flies from the breeding sites in various parts of the country and by tests of resistance in the offspring. The insecticidal effect was usually bioassayed in topical application laboratory tests with field strains of *Musca domestica* and with a susceptible reference strain. The results from our bioassays confirmed that resistance to deltamethrin existed and the resistance of populations collected from Beijing (53- to 250-fold) were higher than that from Tianjin (13- to 67-fold) and Zhangjiakou (51-fold). The PBO synergism experiments indicated a large synergistic effect, i.e., the addition of PBO to deltamethrin caused a greater fly mortality than deltamethrin alone (synergism coefficients ranged from 26- to 156-fold), which suggested the involvement of mixed function oxidases in causing the high levels of deltamethrin resistance seen in these field populations.

*Musca domestica* has long been used as a model insect to study knockdown resistance (Soderlund and Knipple 1999), and based on mutation L1014 F, a simple and reliable cPASA method was used to discern the kdr allele in field house flies populations. The super-kdr point mutation, M918T, can be detected by the multiplex PCR protocol (Li et al. 2003), but in our study, no super-kdr mutation was observed in all field populations or the susceptible Lab strain. By PASA, test kdr allelic frequencies in all field populations ranged from 8 to 56%, susceptible homozygote frequencies were 0–84% (zero in BJF-3), kdr homozygote frequencies were 16–88%, but kdr homozygotes were only found in 10 of 17 of the populations at...
frequencies ranging from 2 to 15%. Thus, \textit{kdr} allele existed mostly in heterozygous and played an important role in causing resistance to deltamethrin. Comparison with Hardy-Weinberg expectations showed a significant excess of heterozygotes in 5 of 17 of the populations. It appears that the target site insensitivity to pyrethroids had a recessive fitness cost causing reduced survival of \textit{kdr} homozygotes. Scott et al. (1997) reported both pupation success and fecundity of \textit{Hematobia irritans irritans} were negatively affected by pyrethroid resistance because of target site abnormalities, which resulted in a decrease of resistance in early season populations before insecticide control was applied. Apart from the sodium channel mutation (\textit{kdr}), PBO synergism test showed that the mechanisms of resistance to deltamethrin in Chinese house flies also include enhanced detoxification by mixed function oxidases. Genetic linkage of insensitive sodium channel and MFO-mediated detoxification has been reported in \textit{H. virescens} (Park and Brown 2001).

We observed no significant differences in the distribution of resistant genotypes between male and female flies in all but one house fly population from Beijing. A positive correlation was found between overall \textit{kdr} frequency and deltamethrin resistance as expressed by the \textit{LD}_{50} value (Fig. 1). Although metabolic mechanisms, such as MFOs, are known to contribute to pyrethroid resistance in pest insects, sodium channel mutations (\textit{kdr}) have been suggested to be the major mechanisms (Guerrero et al. 1997). However, in Coted’Ivoire, a high frequency of \textit{kdr} in \textit{Anopheles gambiae} does not prevent pyrethroid-treated bednets, causing high mortality of these malaria vectors (Asidi et al. 2004).

\textbf{PASA} has been successfully used for detecting the \textit{kdr}-type allele of the \textit{para}-homolog in many insects. A new substitution of the L1014S mutation in the voltage-gated sodium channel gene was identified in Kenyan \textit{An. gambiae} associated with resistance to DDT and pyrethroids (Ranson et al. 2000). Detection of L1014 F and M918T in field \textit{H. irritans irritans} populations was used to investigate the dynamics of pyrethroid resistance (Guerrero et al. 2002a); Phe\textrightarrow Ile amino acid mutation in the sodium channel gene III\textit{S}6 and Asp\textrightarrow Asn mutation in the \textit{CzEst9} esterase were examined to identify possible resistance associated roles of these two amino acid substitutions in \textit{Boophilus microplus} (Guerrero et al. 2002b). The relationship has been determined between the \textit{para}-homologous sodium channel point mutation (g\textrightarrow c at nucleotide 2979) and knockdown resistance in the German cockroach (Valles et al. 2003). The frequency of pyrethroid resistance of the \textit{kdr} type has been used on mosquitoes from three villages in the Bamako and Sikasso areas of Mali as an indication of barriers to gene flow between the Bamako and Savanna chromosomal forms of \textit{An. gambiae} s.s. (Fanello et al. 2003).

A quick PCR-based diagnostic test for the presence of the \textit{kdr} mutation in \textit{M. domestica} would allow its monitoring in natural populations and thus permit better design of insecticide use to improve control programs. However, traditional bioassays are still necessary to evaluate and predict product failure.

Comparison of the PCR-ASO and cPASA showed that the two methods were in agreement in 32 of 33 individuals tested (Fig. 2B, A11). The one case of disagreement was cleared up after retesting with PASA, where a fly which had originally been scored as the SS genotype was identified as SR genotype, in agreement with the result of PCR-ASO. It is considered that the cPASA protocol is accurate enough for practical monitoring of the \textit{kdr}-allele frequencies in house fly populations. ASO-PCR using specific oligonucleotide hybridization was first used for detection mutations in the \textit{betatubulin} gene associated with benzyl resistant in field strains of \textit{Venturia inaequalis} and other pathogenic fungi (Koenraadt et al. 1992). The approach has subsequently been generally applied to the detection of known point mutations underlying several genetic diseases (DeMarchi et al. 1994, Wong and Senadheera 1997). This method has also been called PCR-SSOP (PCR sequence-specific oligonucleotide probing) and has been used in monitoring alleles of the \textit{kdr} gene in \textit{An. gambiae} s.s. (Kolaczinski et al. 2000). Because this technique is relatively simple, sensitive, accurate, and cost-effective (Labuda et al. 1999), it was used to verify the accuracy of the PASA in this study. PCR-ASO is very convenient for processing large numbers of individual samples, especially when several DNA need to be assayed in each sample. This approach has potential for automation with microplates and robotic workstations for high throughput. It can avoid the difficulty in the design of primers and quality control of the PASA technique. However, compared with PASA, PCR-ASO was more time consuming and not so cost-effective, especially in monitoring the single mutation L014 F responsible for knockdown resistance. It can be concluded that cPASA can serve as a practical molecular diagnostic tool to determine the frequency of the \textit{kdr} genotype in wild house fly populations.

\textbf{Acknowledgments}

This study was supported by National Natural Science Foundation of China Grant 39470643. We thank C. Curtis for improving the English of the manuscript.

\textbf{References Cited}

Asidi, A. N., R. N’Guessan, R. A. Hutchinson, M. Traoré-Lamizana, P. Carnevale, and C. F. Curtis. 2004. Experimental hut comparisons of nets treated with carbamate or pyrethroid insecticides, washed or unwashed, against pyrethroid-resistant mosquitoes. Med. Vet. Entomol. 18: 134–140.

Chavasse, D. C., R. P. Shier, O. A. Murphy, S.R.A. Huttly, S. N. Cousens, and T. Akhtar. 1999. Impact of fly control on childhood diarrhoea in Pakistan: community-randomised trial. Lancet. 353: 22–25.

DeMarchi, J. M., C. S. Richards, R. G. Fenwick, R. Pace, and A. L. Beaudet. 1994. A robotics-assisted procedure for large scale cystic fibrosis mutation analysis. Hum. Mutat. 4: 281–290.
Dong, K. 1997. A single amino acid change in the para sodium channel protein is associated with knockdown-resistance (kdr) to pyrethroid insecticides in the German cockroach. Insect Biochem. Mol. Biol. 27: 93–100.

Emerson, P. M., S. W. Lindsay, G. E. L. Walraven, H. Faal, C. Bghk, G. Lowe, and R. L. Bailey. 1999. Effect of fly control on trachoma and diarrhoea. Lancet. 353: 1401–1403.

Fanello, C., V. Petrarca, A. della Torre, F. Santolamazza, G. Dolo, M. Coulhaly, A. Allouche, C. F. Curtis, Y. T. Toure, and M. Coluzzi. 2003. The pyrethroid knock-down resistance gene in the Anopheles gambiae complex in Mali and further indication of incipient speciation within An. gambiae s.s. Insect Mol. Biol. 12: 241–245.

Finney, D. J. 1971. Probit analysis, 3rd ed. University Press, Cambridge, UK.

Greenberg, B. 1965. Flies and disease. Sci. Am. 213: 92–99.

Guerrero, F. D., R. C. Jamroz, D. Kammah, and S. E. Kunz. 1997. Toxicological and molecular characterization of pyrethroid-resistant horn flies, Haematobia irritans idenification of kdr and super-kdr point mutations. Insect Biochem. Mol. Biol. 27: 745–755.

Guerrero, F. D., S. E. Kunz, and D. Kammah. 1998. Screening of Haematobia irritans irritans (Diptera: Muscidae) populations for pyrethroid resistance-associated sodium channel gene mutations by using a polymerase chain reaction assay. J. Med. Entomol. 35: 710–715.

Guerrero, F. D., M. W. Alison, D. M. Kammah, and L. D. Foil. 2002a. Use of the polymerase chain reaction to investigate the dynamics of pyrethroid resistance in Haematobia irritans irritans (Diptera: Muscidae) J. Med. Entomol. 39: 747–754.

Guerrero, F. D., A. Y. Li, and R. Hernandez. 2002b. Molecular diagnosis of pyrethroid resistance in Mexican strains of Boophilus microplus (Acari: Ixodidae). J. Med. Entomol. 39: 770–776.

Huo, X.-B., and X.-J. Wang. 1997. Study on resistance of house fly to chemical insecticides in Shandong Province. Chin. J. Vector. Biol. Contr. 8: 273–274.

Ingles, P. J., P. M. Adams, D. C. Knipple, and D. M. Soderlund. 1996. Characterization of voltage-sensitive sodium channel gene coding sequences from insecticide-susceptible and knockdown-resistant house fly strains. Insect Biochem. Mol. Biol. 26: 319–326.

Iwasa, M., S. Makino, H. Asakura, H. Kobori, and Y. Morimoto. 1999. Detection of Escherichia coli O157:H7 from Musca domestica (Diptera: Muscidae) at a cattle farm in Japan. J. Med. Entomol. 36: 108–112.

Jamroz, R. C., F. D. Guerrero, D. M. Kammah, and S. E. Kunz. 1998. Role of the kdr and super-kdr sodium channel mutations in pyrethroid resistance: correlation of allelic frequency to resistance level in wild and laboratory populations of horn flies (Haematobia irritans). Insect Biochem. Mol. Biol. 28: 1031–1037.

Keiding, J. 1999. Review of the global status and recent development of insecticide resistance in field populations of the house fly, Musca domestica (Diptera: Muscidae). Bull. Entomol. Res. 89: S7–S67.

Koenraadt, H., S. C. Somerville, and A. L. Jones. 1992. Characterization of mutations in the betatubulin gene of benomyl-resistant field strains of Venturia inaequalis and other pathogenic fungi. Phytopathology. 82: 1348–1354.

Kolaczinski, J. H., C. Fanello, J.-P. Hervé, D. J. Conway, P. Carnevale, and C. F. Curtis. 2000. Experimental and molecular genetic analysis of the impact of pyrethroid and non-pyrethroid insecticide impregnated bednets for mosquito control in an area of pyrethroid resistance. Bull. Entomol. Res. 90: 125–132.

Labuda, D., M. Krajnovic, C. Richer, A. Skoll, H. Sinnett, V. Yotova, and D. Sinnett. 1999. Rapid detection of CYP1A1, CYP2D6, and NAT variants by multiplex polymerise chain reaction and allele-specific oligonucleotide assay. Anal. Biochem. 275: 84–92.

Lee, S. H., T. J. Smith, D. C. Knipple, and D. M. Soderlund. 1999. Mutations in the house fly Vssl sodium channel gene associated with super-kdr resistance abolish the pyrethroid sensitivity of Vssl/tip E sodium channels expressed in Xenopus oocytes. Insect Biochem. Mol. Biol. 29: 185–194.

Li, A. Y., F. D. Guerrero, C. A. Garcia, and J. E. George. 2003. Survey of resistance to permethrin and diazinon and the use of a multiplex polymerase chain reaction assay to detect resistance alleles in horn fly, Haematobia irritans irritans (L.) J. Med. Entomol. 40: 942–949.

Lin, L. F., Z. H. Zhang, L. P. Liu, X. D. Huang, Z. N. Liang, and R. F. Hao. 1999. Resistance of Musca domestica and its management strategies. Chin. J. Vector Biol. Contr. 10: 21–23.

Liu, D. L., and D. P. Dong. 2001. An analysis of the resistance house fly in Hubei Province from 1991 to 1998. Chin. J. Vector Biol. Contr. 12: 345–346.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darrriet, J. B. Bergé, A. L. Devonshire, P. Guillett, N. Pasteur, and D. Pauron. 1998. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. Insect Biochem. Mol. Biol. 7: 179–184.

Martinez-Torres, D., S. P. Foster, L. M. Field, A. L. Devonshire, and M. S. Williamson. 1999a. A sodium channel point mutation is associated with resistance to DDT and pyrethroid insecticides in the peach-potato aphid, Myzus persicae (Sulzer) (Hemiptera: Aphiidae). Insect Mol. Biol. 8: 339–346.

Martinez-Torres, D., C. Chevillon, A. Brun-Barale, J. B. Bergé, and D. Pauron. 1999b. Voltage-dependent Na+ channels in pyrethroid-resistant Culex pipiens L. mosquitoes. Pestic. Sci. 55: 1012–1020.

Milani, R. 1954. Comportamento mendeliano della resistenza alla azione abbattitante del DDT: correlazione tra abbattimento emortalità in Musca domestica L. Riv. Parasitol. 15: 513–542.

Miyazaki, M., K. Ohyama, D. Y. Dunlap, and F. Matsumura. 1996. Cloning and sequencing of the para-type sodium channel gene from susceptible and kdr-resistant German cockroaches (Blatella germanica) and house fly (Musca domestica). Mol. Gen. Genet. 252: 61–68.

Park, S., and T. M. Brown. 2001. Linkage of genes for sodium channel and cytochrome P450 (CYP6B10) in Heliotis virescens. Pest Manag. Sci. 57: 209–212.

Ransome, J., I. M. Vujcic, X. Wang, J. Hemingway, and F. H. Collins. 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol. Biol. 9: 491–497.

Raymond, M., and F. Bousses. 2003. An update version of GENPOP. v. 1.2 described in: population genetics software for exact tests and ecumenicism. J. Heredity. 86: 248–249.

Raymond, M., G. Prato, and D. Ratsiri. 1993. PROBIT analysis of mortality assays displaying quantal response, version 3.3. Praxeme, St. Georges d'Orques, France.

Rice, W. R. 1989. Analysing tables of statistical tests. Evolution. 43: 223–225.
Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: new computer program for probit analysis. Bull. Entomol. Soc. Am. 23: 209–213.

Sawicki, R. M. 1978. Unusual response of DDT-resistant houseflies to carbinol analogues of DDT. Nature. 275: 443–444.

Schuler, T. H., D. Martinez-Torres, A. J. Thompson, I. Denholm, A. L. Devonshire, I. R. Duce, and M. S. Williamson. 1998. Toxicological, electrophysiological, and molecular characterization of knock-down resistance to pyrethroid insecticides in the diamondback moth, Plutella xylostella (L.). Pestic. Biochem. Phys. 59: 169–192.

Scott, J. A., F. W. Plapp, and D. E. Bay. 1997. Pyrethroid resistance associated with decreased biotic fitness in horn flies (Diptera: Muscidae). Southwest. Entomol. Rep. 22: 405–410.

Scott, J. G., T. G. Alefantis, P. E. Kaufman, and D. A. Rutz. 2000. Insecticide resistance in house flies from caged-layer poultry facilities. Pest Manag. Sci. 56: 147–153.

Smith, T. J., S. H. Lee, P. J. Ingles, and D. C. Knipple. 1997. The L1014F point mutation in the house fly Vssc1 sodium channel confers knockdown resistance to pyrethroids. Insect Biochem. Mol. Biol. 27: 807–812.

Soderlund, D. M., and D. C. Knipple. 1999. Knockdown resistance to DDT and pyrethroids in the house fly (Diptera: Muscidae): from genetic trait to molecular mechanism. Ann. Entomol. Soc. Am. 92: 909–915.

Sun, C.-X., and J.-L. Fan. 1993. Comparison of the toxins for beta-cypermethrin and cypermethrin to house fly Musca Domestica L. and mosquito Culex pipiens pallens Chin. J. Vector Biol. Contr. 4 : 52–54.

Tan, J., T. D. Liu, S. M. Valles, A. L. Goldin, and K. Dong. 2002. Novel sodium channel gene mutations in Blattella germanica reduce the sensitivity of expressed channels to deltamethrin. Insect Biochem. Mol. Biol. 32: 445–454.

Vais, H., M. S. Williamson, C. A. Hick, N. Eldursi, A. L. Devonshire, and P.N.R. Usherwood. 1997. Functional analysis of a rat sodium channel carrying a mutation for insect knock-down resistance (kdr) to pyrethroids. FEBS Lett. 413: 327–332.

Valles, S. M., O. P. Perera, and C. A. Strong. 2003. Relationship between the para-Homologous sodium channel point mutation (g→c at Nucleotide 2979) and knockdown resistance in the german cockroach using multiplex polymerase chain reaction to discern genotype. J. Econ. Entomol. 96: 885–891.

Williamson, M. S., I. Denholm, C. A. Bell, and A. L. Devonshire. 1993. Knockdown resistance (kdr) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the house fly (Musca domestica). Mol. Gen. Genet. 240: 17–22.

Williamson, M. S., D. Martinez-Torres, C. A. Hick, and A. L. Devonshire. 1996. Identification of mutations in the house fly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Mol. Gen. Genet. 252: 51–60.

Wong, L.J.C., and D. Senadheera. 1997. Direct detection of multiple point mutations in mitochondrial DNA. Clin. Chem. 43: 1857–1861.

Zhang, A. G., J. B. Dunn, and J. M. Clark. 1999. An efficient strategy for validation of a point mutation associated with acetylcholinesterase sensitivity to azinphosmethyl in Colorado potato beetle. Pestic. Biochem. Physiol. 65: 25–35.

Zhu, Y. C., K. J. Kramer, B. Oppert, and A. K. Dowdy. 2000. cDNA of aminopeptidase-like protein genes from Plodia interpunctella strains with different susceptibilities to Bacillus thuringiensis toxins. Insect Biochem. Mol. Biol. 30: 215–224.

Received for publication 20 January 2005; accepted 25 July 2005.