Pesticide Biochemistry and Physiology 105 (2013) 161–168

Contents lists available at SciVerse ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

# Variable fitness costs for pyrethroid resistance alleles in the house fly, *Musca domestica*, in the absence of insecticide pressure

Frank D. Rinkevich, Cheryl A. Leichter, Tomás A. Lazo, Melissa C. Hardstone, Jeffrey G. Scott *

*Department of Entomology, Cornell University, Comstock Hall, Ithaca, NY 14853 0901, USA*

ARTICLE INFO

*Article history:*
Received 1 November 2012
Accepted 18 January 2013
Available online 5 February 2013

*Keywords:*
Sodium channel
Cytochrome P450
*Super-kdr*
Pyrethroid resistance
House fly
Fitness costs

ABSTRACT

Resistance to pyrethroids is caused by mutations in the voltage-sensitive sodium channel (*Vssc*) and enhanced metabolic detoxification by CYP6D1 in the house fly, *Musca domestica*. We investigated if there was a fitness cost associated with resistance alleles by performing a competition study with multiple *Vssc* and *CYP6D1* alleles under laboratory conditions in the absence of insecticides. The *kdr1* haplotype is significantly favored over the *kdr2*, *super-kdr* and susceptible *v3* haplotypes. The initial frequencies of *kdr2*, *super-kdr* and *v3* declined and remained low through the course of the experiment. The *v5* and *v6* haplotypes did not change from their initial frequency and were considered to be neutral. Genotypes containing either *kdr2* or *v3* became increasingly rare despite being the most frequent genotypes at the initiation of the experiment. Heterozygotes containing *kdr1* and *kdr1/kdr1* homozygotes accounted for the majority of genotypes after the $F_1$. The susceptible *v5* and *v6* haplotypes were mostly found as heterozygotes with *kdr1*, and any combination of *v5* and *v6* did not exceed 5% at any generation. This suggests that *kdr1* carries a fitness advantage and *kdr2*, *super-kdr* and *v3* are at a fitness disadvantage under the environmental conditions of this experiment. The frequency of the resistant *CYP6D1v1* allele increased over the course of the experiment, but did not deviate significantly from HWE. Thus, there is no fitness cost for the *CYP6D1v1* allele under these conditions. These results are compared to previous research on field collected populations, and the impact of the fitness advantage of an insecticide resistance allele on insecticide resistance evolution and management is discussed.



## 1. Introduction

House flies (*Musca domestica*) are cosmopolitan pests that transmit numerous pathogens including the causative agents of cholera, trachoma, and salmonellosis [1]. They have also been known to transmit the lethal O157:H7 strain of *Escherichia coli* in both Japan and the United States [2] and antibiotic resistant bacteria [3]. House flies can vector *Yersinia pseudotuberculosis* [4], which can result in high avian mortality on poultry farms [5]. House fly infestations cause lowered agricultural production, reduced feed conversion efficiency, and increased stress levels for young or adult animals, leading up to $200 M in annual production losses [6].

Pyrethroids such as permethrin, cyfluthrin and deltamethrin, are widely used throughout the United States for effective pest control due to their high levels of efficiency and low mammalian toxicity [7,8]. Pyrethroids act on voltage-sensitive sodium channels (*Vssc*) [9], and only a small proportion of sodium channels (3–4%) need to be affected by pyrethroids to cause mortality [10,11].

The major mechanisms of pyrethroid resistance in the house fly are target site insensitivity and increased metabolic detoxification by cytochrome P450s [12]. The *kdr* (L1014F mutation in *Vssc*) and *super-kdr* alleles (M918T + L1014F mutations) confer resistance [13] by making a channel that is much less sensitive to pyrethroids (due to modified channel gating kinetics) [14,15]. Overexpression of *CYP6D1* leads to metabolism-mediated pyrethroid resistance [16]. There is only one *CYP6D1* resistance allele (*CYP6D1v1*) [17].

The appearance of a resistance allele, while having a selective advantage in the presence of insecticide, often has a fitness disadvantage or cost, in the absence of insecticide use under field conditions [18–20]. A fitness cost can be manifest in a myriad of ways: Increased overwintering mortality [21–23], increased likelihood of being caught by predators [24], lower reproductive output [24], altered development time [25], lower energy reserves [25], etc. However, the premise of a single resistant (R) and single susceptible (S) haplotype is a gross oversimplification, as multiple R and S haplotypes occur in many cases [26–29]. What is lacking is an understanding of the relative fitness costs for each allele and/or haplotype. Do all R alleles or haplotypes of a gene behave the same (favored in the presence of insecticide and costly in the absence of insecticide)? Do all S haplotypes behave the same?

Relatively little is known about the fitness costs (in the absence of insecticide use) of *Vssc* and *CYP6D1* resistance alleles. The frequency of these resistance alleles in house flies increased over

* Corresponding author. Tel.: +1 607 255 7340; fax: +1 607 255 0939.
*E-mail address:* jgs5@cornell.edu (J.G. Scott).

0048-3575/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pestbp.2013.01.006

the course of a field season in New York when insecticides were being used, but declined over the winter, suggesting a fitness cost to these resistant alleles [23]. Laboratory studies have found that house flies with *kdr* did not show the same preference for warmer temperatures as was found for susceptible flies [30], and that *kdr* resistant aphids were significantly less likely to respond to increasing amounts of alarm pheromone [31].

The goal of this study was to monitor the changes in *Vssc* and *CYP6D1* alleles over 30 generations in a controlled no insecticide environment. As expected, two *Vssc* resistance alleles/haplotypes (*kdr2* and *super-kdr*) declined in frequency over time. Unexpectedly, the *kdr1* haplotype increased in frequency. Two of the S haplotypes (*v5* and *v6*) remained relatively unchanged over the 30 generations, while *v3* decreased. There were no significant changes in the frequency of *CYP6D1v1*. The implications of these results to resistance monitoring are discussed.

## 2. Materials and methods

### 2.1. House fly strains

Two strains of house flies, *M. domestica L.*, were used. CPR is a heterozygous pyrethroid resistant strain (due to *kdr*, *super-kdr* and *CYP6D1v1*) originally from NY. Sma is an insecticide susceptible strain (although it has a very low frequency of *kdr*) with morphological markers on each of the five autosomes. Flies were reared at 28 °C with 40% RH and 12:12 light:dark photoperiod as previously described [32]. Female Sma flies ($n = 435$) were crossed with male CPR flies ($n = 200$). The $F_1$ generation was then randomly split into three different replicates (A, B and C). Each replicate was then allowed to freely interbreed for 30 generations. Approximately 800–1000 flies were present in each cage for each generation. Individual male flies were collected at generations 1, 10, 20 and 30 in 1.5 ml tubes containing 750 μl of 70% ethanol and stored at −74 °C and genotyped as described below.

### 2.2. Genotyping and analysis of Vssc sequences

A 335 bp fragment of *Vssc* was amplified by PCR from genomic DNA as previously described [28] to determine the haplotype(s) present. Sequencing was performed at Cornell's Biotechnology Resource Center. Electropherograms were inspected for the *Vssc* alleles present in each sample. Individual animals were determined to be homozygous, or heterozygous for *kdr* based on the 1014 site in the sequence (codons CTT = susceptible and TTT = resistant). Individuals having a L1014F mutation were also genotyped for the *super-kdr* mutation as described previously [28]. The specific susceptible (*v3*, *v5* and *v6*) and resistant (*kdr1* and *kdr2*) haplotypes were determined by comparing the sequence of the intron directly adjacent to the 1014 codon [28]. Each sequence was resolved by inspecting electropherograms and comparing the sequences to known alleles from the CPR and Sma strain that were used in the initial cross.

### 2.3. Genotyping and sequencing of CYP6D1

A fragment of *CYP6D1* was amplified with the primer pair of S23 (5′-TATGGCATGACGTTGAGTCG-3′) and AS6 (5′-CAGTTTTGTGTCG GGTACTTG-3′). Reagent mixtures contained ReddyMix Master Mix (ABgene House, Surrey, UK) or GoTaq Green Master Mix (Promega, Madison, WI). For both Master Mix products the following thermal cycler program was used: 95 °C for 5 min followed by 35 cycles of 95 °C for 30 s, 47 °C for 30 s, and 72 °C for 1 min and a final extension of 72 °C for 10 min. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA), and sequenced at Cornell's Biotechnology Resource Center. The *CYP6D1* genotype was determined by manual inspection of the sequence for the 15 bp insert in the 5′ promoter region that is indicative of *CYP6D1v1* [26].

### 2.4. Data analysis

Hardy–Weinberg equilibrium calculations were performed for two alleles (R and S, for both *Vssc* and *CYP6D1*) and six haplotypes (*v3*, *v5*, *v6*, *super-kdr*, *kdr1*, and *kdr2*). Deviations from Hardy–Weinberg equilibrium were analyzed with a $\chi^2$ test ($p < 0.05$). To account for multiple testing, we used the Bonferroni correction. Using Fisher's combined probabilities test we determined a global *p*-value for the set of Hardy–Weinberg equilibrium tests. Significant differences in allele (or haplotype) and genotype frequencies between generations were analyzed with a $2 \times 2$ $\chi^2$ contingency table with the Fisher's Exact Test ($p < 0.05$) for each possible comparison of alleles or genotypes at each generation.

The probability of allele or haplotype frequency changes due to genetic drift acting alone between generations was simulated in R (http://cran.r-project.org/) and calculated from the binomial distribution. The simulation assumed a panmictic population with fixed size of 200 diploid individuals with allele frequencies defined by the empirical observation at the start of the interval. For each generation, half of the alleles from the population were sampled at random and from this group the next generation allele frequencies were determined. This process was repeated for the number of generations within the interval of interest. *p*-Values were obtained by dividing the number of simulations that resulted in an allele frequency change as great as or greater than the observed R allele frequency at the end of the interval of interest by the 10,000 total simulations run. The average of five *p*-values was reported and subjected to Bonferroni correction. Fisher's combined probabilities test was used to determine a global *p*-value for the set of genetic drift tests.

For the determination of R allele (or haplotype) frequency changes over time, all replicates were combined ($n = 3$). Statistical differences between means were determined using Tukey's test ($p < 0.05$).

## 3. Results

### 3.1. Vssc alleles/haplotypes

Three different *Vssc* alleles were present in the parental strains of house flies: *kdr*, *super-kdr*, and susceptible. There were two *kdr* haplotypes (*kdr1* and *kdr2*), one *super-kdr* haplotype and three susceptible haplotypes (*v3*, *v5* and *v6*) found. As expected, all of these haplotypes were found in the $F_1$ (Tables 1 and 2, Fig. 1).

If alleles are grouped as either resistant (*kdr* or *super-kdr*) or susceptible (L1014), they are in Hardy–Weinberg Equilibrium (HWE) in each replicate at generations 10–30 (Table 1). Therefore, there is no apparent difference in the fitness of resistant versus susceptible *Vssc* alleles under these conditions using these criteria.

In contrast to simple comparisons of resistant and susceptible alleles, when all six *Vssc* haplotypes are considered, there were clear fitness differences observed and the population is not in HWE (Table 2). For the resistant haplotypes, the frequency of *kdr1* increased over 30 generations, approaching a plateau at a frequency of about 0.6 (Fig. 1). The fitness advantage of *kdr1* was manifest fairly quickly, being a frequency of nearly 0.5 by the $F_{10}$ (Table 2). Conversely, the *kdr2* haplotype decreased rapidly and significantly over the 30 generations. By $F_{10}$, the frequency of *kdr2* had decreased significantly to 0.06 (from 0.24 in the $F_1$) and further decreased to 0.03 at generation 30 (Fig. 1). The *super-kdr*