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Effects of artificial migration of susceptible individuals on resistance and fitness of a fenitrothion-resistant strain of *Musca domestica* (L.) Diptera

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Abstract: Migration of susceptible individuals from untreated areas to populations that have developed insecticide resistance is one of the most important processes that can significantly delay or even prevent the development of resistance against insecticides. Fitness parameters of susceptible and resistant insect populations and changes in fitness following susceptible population migration have a crucial place in this process, as they determine the permanence and spread of susceptible alleles in the absence of insecticides. In this study, we investigated changes in resistance levels and fitness characteristics after introducing individuals from the ancestral susceptible strain in an equal ratio to a housefly (*Musca domestica*) strain artificially selected against fenitrothion, an organophosphate insecticide. We measured fitness parameters such as pre-adult development time, fecundity, fertility, and survival. Compared to the susceptible strain, the resistant strain had slower development time, but there were not any significant differences for fecundity, fertility, and survival. The level of resistance decreased gradually with 2 generations of susceptible migration. Development times were faster in both migration strains compared to the resistant strain. In addition, we detected a fitness reduction in fecundity, fertility, and female survival after the first generation of migration, but this reduction was alleviated after the second generation of migration. In conclusion, these findings indicate that fenitrothion resistance in *Musca domestica* has important fitness costs related with development time, and these costs are mitigated with susceptible migration.

Key words: Insecticide resistance, fitness, susceptible, fenitrothion, migration, *Musca domestica*

1. Introduction

One of the most important anthropogenic-based examples of natural selection is the development of resistance against insecticides. The origins, spread, and mechanisms of insecticide resistance have importance in both theoretical and practical issues (Hemingway, 2000; ffrench-Constant et al., 2004). From the beginning of the first insecticide treatment programs against insect pests, many insect species developed significant resistance levels against insecticides, and the number of resistant populations is still increasing (Georghiou, 1994; Denholm et al., 2002; Hemingway et al., 2002; Hardstone and Scott, 2010). Resistance against pesticides is seen as the product of 2 interacting forces. These are selection pressure acting on different genotypes in the presence or absence of the selecting agent (the insecticide) and gene flow, usually within a Mendelian population (May and Dobson, 1986).

In order to overcome the development of resistance, several resistance-management programs are proposed, like utilization of synergists that inhibit the resistance mechanisms, managing the dominance of the resistant alleles by saturation, rotation of different types of insecticides, and provision of untreated refuges to preserve susceptible alleles (Roush, 1989; Georghiou, 1994; Lenormand and Raymond, 1998). Management strategies that utilize the untreated zones to allow continuous migration of susceptible individuals to resistant populations should take into consideration the relative fitness of resistant alleles, as relative fitness is one of the main factors that determine the dynamics of resistant alleles in the absence of insecticides (Crow, 1957; May and Dobson, 1986; McKenzie and Clark, 1988; Roush, 1989; Minkoff and Wilson, 1992; McKenzie, 2000; Boivin et al., 2001; Haubruge and Arnaud, 2001).

Crow (1957) first pointed out that resistant and susceptible strains differ in fitness characteristics, such as development time, fecundity, and fertility. It is also generally assumed that resistant genotypes must have pleiotropic effects that result in reproductive disadvantage relative to susceptible genotypes, because in the absence of pesticides (i.e. selection agents), the resistant types are not common in pest populations before selection.

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If the selective pressure is relaxed because of stabilizing selection, resistance alleles will decline in frequency (Crow, 1957; Roush and McKenzie, 1987; Carriere et al., 1994; McKenzie, 2000; Shi, 2004). By measuring reproductive, developmental, and behavioral fitness components of numerous resistant insect species, many studies have recorded the fitness costs of resistance alleles in the absence of insecticide selection pressure (Clarke and McKenzie, 1987; Rowland, 1991a, 1991b; Minkoff and Wilson, 1992; Boivin et al., 2001; Boivin et al., 2003; Foster et. al., 2003; Bourguet et al., 2004; Liu and Han, 2006).

The housefly, *Musca domestica* L. (Diptera: Muscidae), is an important mechanical vector of both human and animal diseases. The housefly's insecticide resistance has become a global problem, as it has developed resistance against almost every insecticide used against it (Georghiou and Mellon, 1983; Scott et al., 1989; Kristensen, 2000; Acevedo et al., 2009; Kaufman, 2010; Memmi, 2010). In addition, because of its high potential for insecticide resistance, *Musca domestica* is also a suitable model for studying the genetic and metabolic mechanisms of insecticide resistance. Several mutations conferring insecticide resistance have been defined for *Musca domestica*. These include insensitive acetylcholinesterases (AChEs) (Bourguet et al., 1997; Kozaki et al., 2001; Walsh et al., 2001; Fournier, 2005) and altered glutathione S-transferases conferring organophosphate resistance (Wei et al., 2001; Enayati et al., 2005; Kristensen, 2005), mutations in cytochrome P450 (Feyereisen et al., 1989; Tomita, 1995; Scott, 1999; Seifert and Scott, 2002), and kdr and super-kdr mutations (Miyazaki, 1996; Williamson, 1996) in pyrethroid resistance.

In this study, we designed an experiment to test the effects of susceptible migration to a resistant strain on resistance level and fitness traits in housefly, *Musca domestica*. First, we provided artificial selection for 5 generations with fenitrothion on a laboratory population (GS) that had been sampled from Gaziantep, and we obtained a fenitrothion-resistant (GFR) strain. We then combined individuals from the ancestral susceptible (GS) and resistant (GFR) strains in an equal proportion to simulate the reintroduction of susceptible individuals and after oviposition obtained the first migration strain (GFM-1). The same procedure was repeated by introducing susceptible individuals from the GS strain to GFM-1 strain, and the second migration strain, GFM-2, was obtained. We used the unselected ancestral strain (GS) as the source of susceptibles to minimize other differential factors related to genetic background that can influence fitness.

Resistance levels and life history parameters like pre-adult development time, fecundity, fertility, and survival were compared between all strains to test for related changes in fitness parameters and insecticide resistance levels in response to susceptible migration.

2. Materials and methods

2.1. Housefly strains and insecticides

The GS strain was a laboratory-adapted strain obtained from a garbage dump area in Gaziantep Province in southeastern Turkey. The strain was reared at Hacettepe University Ecological Sciences Research Laboratory for 70 generations. Insecticide usage was high at the sampling site, including cypermethrin, permethrin, and fenthion formulations. The WHO (F_{178}) strain is a standard insecticide-susceptible strain obtained from the World Health Organization (WHO) and bred in the laboratory for 178 generations.

Flies were reared in the laboratory under a 12/12 h light/dark photoperiod, 25 ± 2 °C temperature, and 70 ± 5% relative humidity (RH). After the fourth day of adult emergence, cotton soaked in milk powder and water was placed in the cages (30 × 30 × 30 cm). Larvae were reared in jars (1000 cm^3) using a mixture of water, milk powder, and bran as the standard growth medium (500 g wheat bran, 120 g milk powder, and 500 mL water). Larval density was balanced to be 180 larvae per 18 g medium, as modified from Çağlar (1991) and Farnham (1984). For adult emergence, pupae were placed in separate cages.

An organophosphate insecticide, fenitrothion, was used in bioassays and selections.

2.2. Bioassays and resistance selection

Bioassays were conducted with 1-day-old, adult, and virgin females that were separated within 12 h after emergence from pupae (housefly adults begin mating 12 h after emergence). Fenitrothion was diluted with acetone. In the control groups 1 µL of acetone and in the bioassay groups 1 µL of acetone + insecticide were applied topically to the mesothorax with a microapplicator (Burkard Scientific) (Fisk and Isert, 1953; Collins, 1975).

Insecticide resistance bioassays were carried out with 3 repeats of 20 individuals per insecticide dosage, totaling 60 individuals per dosage. In order to assess the insecticide resistance levels, 5 and 4 dosages were used. Fenitrothion dosages used in insecticide bioassays were determined according to Akiner and Çağlar (2006) and are shown in Table 1. Individuals used in insecticide assays were fed before and after application (provided with cotton soaked in sugar and water solution after topical application); individuals treated with insecticides by topical application were maintained in the same conditions as stock strains (i.e. 12/12 h L/D photoperiod, 25 ± 2 °C, and 70 ± 5% RH). The survival of treated insects was recorded after 24 h, and LD_{50} values were determined according to Finney (1952). Resistance ratios were estimated relative to the WHO susceptible strain.

Table 1. Fenitrothion dosages (g/mL) used in insecticide bioassays.

	1	2	3	4	5
WHO	0.32×10^{-5}	1.6×10^{-5}	8×10^{-5}	4×10^{-4}	20×10^{-4}
GS	1.6×10^{-5}	8×10^{-5}	4×10^{-4}	2×10^{-3}	10×10^{-3}
GFR	4×10^{-4}	1×10^{-3}	2×10^{-3}	5×10^{-3}	10×10^{-3}
GFM-1	8×10^{-4}	4×10^{-4}	1×10^{-3}	2×10^{-3}	10×10^{-3}
GFM-2	8×10^{-4}	5×10^{-3}	10×10^{-3}	20×10^{-3}	–

For resistance selection, females and males from the GS strain that survived applications of greater than the LD_{50} level were placed in separate cages for oviposition, and oviposited eggs were collected daily. Individuals emerging from these eggs were used to construct the F_1 generation. We repeated this procedure continuously for 5 generations to obtain the fenitrothion-resistant GFR strain.

2.3. Susceptible migration

For experimentally simulating a continuous susceptible migration to a resistant population, we used the following procedure. In order to obtain mixed populations of susceptible and resistant individuals, 25 virgin (separated before 12 h) males and 25 virgin females from the susceptible GS strain and 25 virgin males and 25 virgin females from the resistant GFR strain were combined to create a cohort of 100 individuals. After mating and oviposition, the eggs were collected and used to obtain the first migration strain, GFM-1. The same procedure was repeated by combining individuals from the GS strain and GFM-1 without interval, and after mating and oviposition we obtained the second migration strain, GFM-2.

2.4. Fitness assays

To obtain cohorts consisting of adults that emerged in the same period, cages were checked daily for adult emergence, and adults emerging before 12 h were separated and life history parameters were recorded. Each cohort initially consisted of 50 virgin females and 50 virgin males. One cohort per strain was used for fitness assays.

The following parameters were recorded daily: number of live females, number of live males, number of oviposited eggs, date of pupation, and date of emergence of pupae. After counting, the eggs were placed in jars with larval medium. Close to pupation, dry bran was placed on top of the larval medium to provide a suitable environment for pupation. For each jar, we checked daily for new pupae, and new pupae were separated and placed in cages for adult emergence. New emerging adults were counted daily starting from the first day of emergence.

For each strain we constructed daily; schedules of eggs laid; from these schedules, lifetime fecundity was calculated as eggs laid per live female, and lifetime fertility was calculated as adults emerging from eggs per live females during the oviposition period. Egg production was followed for 30 days for each cohort, as by this time females of all strains had ceased egg laying. Pre-adult development time was taken as the period between emergence from egg to emergence from pupae. Fitness parameters were measured for the F_1 generations of resistant (GFR) and migration (GFM-1 and GFM-2) strains and for the F_{70} generation of the susceptible GS strain.

2.5. Statistical analysis

LD_{50} values of strains were assessed with the EPA Probit Analysis Program, v. 1.5. Total pre-adult development times of resistant strains were compared with one-way ANOVA with Statistica, v. 7. Pre-adult development time data were log_{10}-transformed before analysis. Survival times between different populations were estimated with Kaplan–Meier survival analysis and compared with log-rank test (SPSS 15.0 for Windows). Fecundity and fertility patterns were compared between strains with one-way ANOVA testing with Statistica v. 7; both fecundity and fertility values were log(ln)-transformed before analysis.

3. Results

3.1. Resistance levels

At the beginning of selection, the level of resistance to fenitrothion in the GS strain was 7.93-fold compared with the standard susceptible WHO strain. After 5 generations of selection, the resistance ratio increased to 223-fold (Table 2) in the GFR strain. This indicates that after 5 generations of selection, the degree of development of resistance in the resulting fenitrothion-resistant GFR strain was 28.12-fold that of the parental GS strain.

After the first generation of susceptible (GS) migration to the GFR strain, the level of the resistance ratio dropped to approximately 83 in the GFM-1 strain compared to the WHO strain. After the second susceptible migration, the resistance ratio dropped to 45 in the GFM-2 strain. Thus, with continuous susceptible migration, after the first migration the level of resistance decreased to 37%, and then in the second migration to 23% of the resistance level of the resistant GS population (Table 2).

Table 2. LD_{50} values for all strains.

	Fenitrothion			
Fly strain	Slope ± SE	Chi-square	LD_{50} (95% CL)	RR[a]
WHO	1.13 ± 0.12	2.98	1.30 (0.84–1.90)	1
GS	1.32 ± 0.13	1.54	10.31 (7.23–14.35)	7.93
GFR	3.49 ± 0.33	4.90	290.06 (252.23–333.9)	223
GFM-1	4.79 ± 0.99	8.20	108.89 (69.59–160.21)	83.76
GFM-2	3.50 ± 0.53	4.80	58.691 (47.51–69.03)	45.14

RR[a] = Resistance ratio relative to WHO susceptible strain. LD_{50} values are $\times 10^{-5}$ (g/mL).

3.2. Development time

Pre-adult development time values for all strains are shown in Table 3 and the Figure. The susceptible GS strain had the shortest pre-adult development time, with the GFM-2 strain following it, and the longest pre-adult development time was in the GFR strain. When all strains were compared together for pre-adult development time with one-way ANOVA, there were significant differences among strains ($P < 0.01$). Post-hoc tests revealed that differences were significant among all strains for pre-adult development ($P < 0.01$).

3.3. Fecundity and fertility

Fecundity and fertility values are shown in Table 3. The GFM-1 strain had the lowest fecundity. When fecundity parameters were compared for all strains with one-way ANOVA, there were significant differences among all strains ($P < 0.01$). Post-hoc tests revealed that when compared pairwise, the differences among GS, GFR, and GFM-2 strains were not significant ($P > 0.05$), but differences were significant between GFM-1 and all other strains for pairwise tests ($P < 0.05$). As with fecundity, there were significant differences in fertility among all strains when they were compared together ($P = 0.02$). Post-hoc tests revealed that the GFM-1 strain was significantly different from the GS and GFR strains ($P < 0.05$). In addition, GFM-1 had the lowest fertility.

3.4. Survival

For female survival distributions, there was significant difference when all strains were compared together (chi-square = 13.52, df = 3, $P < 0.01$). When we compared the survival distributions pairwise, survival values for GFM-1 females were significantly lower than all other strains ($z = 2.61$, $P = 0.009$ for GS GFM-1; $z = 3.32$, $P < 0.001$ for GFR GFM-1; $z = -2.03$, $P = 0.042$ for GFM-1 GFM-2) ($P < 0.05$). There was also significant difference between GFR and GFM-2 ($z = 2.14$, $P = 0.03$) (Table 4).

For male survival, the difference among all strains was significant when they were compared together (chi-square = 27.24, df = 3, $P < 0.001$). For pairwise tests, there was no significant difference between the GFM-1 and GFM-2 strains ($z = 1.02$, $P = 0.23$), but all other comparisons showed significant difference ($P < 0.05$). Interestingly, male survival had the lowest value in the GS strain (Table 5).

4. Discussion

According to Akiner and Çağlar (2006), fenitrothion formulations have been used in Turkey since the 1980s, but their usage began to decline in the 1990s. Fenitrothion resistance was first recorded by Taylor (1982) and by Sisli et al. (1983) in Turkey. Baskurt et al. (2011) reported that frequencies of AChE mutations causing resistance against organophosphates are common in southern regions of Turkey, which includes the sampling location of the ancestor GS strain used in this study.

After resistance selection, the resistance ratio climbed to 223-fold in the GFR strain compared to 7.93-fold in the GS strain. With continuous susceptible migration, resistance level dropped gradually in the GFM-1 and GFM-2 populations (Table 2). However, the level of resistance was still formidable (5.7-fold compared to GS strain) in

Table 3. Lifetime fecundity, fertility, and development (dt) time parameters for all strains. In each row, figures which share the same letter don't differ significantly for pairwise comparisons (post-hoc tests).

	GS	GFR	GFM-1	GFM-2	df	F	P
Mean fecundity ± std. err.	17.83 ± 2.76[a]	21.43 ± 4.52[a]	4.01 ± 1.58[b]	16.73 ± 3.07[a]	3	5.36	<0.01
Mean fertility ± std. err.	2.85 ± 0.81[a]	2.7 ± 0.97[a]	0.62 ±0.24[b]	2.1 ± 0.48[a,b]	3	3.57	0.02
Mean pre-adult dt ± std. err.	9.94 ± 0.02[a]	11.64 ± 0.01[b]	10.62 ± 0.03[c]	11.1 ± 0.01[d]	3	1641	<0.01

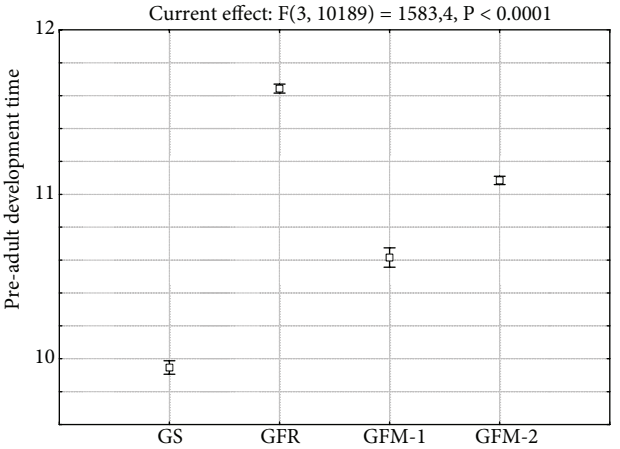

Figure. Mean pre-adult development time (in days) values for all strains. Vertical bars denote 0.95 confidence intervals.

the GFM-2 strain after 2 generations of migration. It is remarkable that we produced a relatively high migration rate of 50%, considering that the rate of migration is much lower in fields after insecticide applications.

Regarding the fitness costs, many studies reported the deleterious pleiotropic effects of insecticide resistance on several life history traits affecting fitness (Roush and Plapp, 1982; Minkoff and Wilson, 1992; Carriere et al., 1994; Zhu et al., 1996; Boivin et al., 2001; Bourguet et al., 2004). For development time, our results show that the fenitrothion-resistant strain (GFR) has a significantly longer pre-adult development time compared with the parental GS strain (Table 3). This delay in the development time of the resistant strain points to an important trade-off between resistance and fitness, as development time is an important life-history component of fitness (Roff, 1992; Stearns, 2000), and small deviations in development time would have more impact on population growth rate relative to similar degrees of changes in fecundity (Roush and Croft, 1986). With susceptible migration to the resistant population, this delay in the development time shortened relative to the resistant strain, as both GFM-1 and GFM-2 strains had significantly lower development times compared to the GFR strain (Figure; Table 3). However, development time was longer in the GFM-2 strain relative to GFM-1, so this would indicate that fitness would show a fluctuating change after susceptible migration. For captive laboratory populations, reduced fitness is a common condition due to inbreeding and genetic drift (Shabalina et al., 1997; Reed and Bryant, 2000). However, in our study, the resistant strain (GFR) and the susceptible parental strain (GS) had the same genetic background. Thus, it is probable that the observed difference in development time of resistant and susceptible strains is due to the pleiotropic effects of insecticide resistance. If resistance is caused by mutations conferring insensitivity of the target site of pesticides, a fitness trade-off would probably involve parameters like development time, which depends more on neural regulating mechanisms, compared to parameters like fecundity or fertility, which rely more on allocation processes (Williamson et al., 1996; Foster et al., 2003).

Considering reproductive parameters, we did not observe any significant difference between resistant GFR and susceptible GS strains for overall lifetime fecundity and fertility. The differences between susceptible GS and resistant GFR strains were not significant for life-time fecundity and fertility, as shown in Table 3. This could mean that the development of fenitrothion resistance does not have a trade-off related to reproductive parameters. Many studies have shown that there is little or no fitness difference between resistant and susceptible strains for some resistant insect strains (Baker et al., 1998; Haubruge and Arnaud, 2001, 2002, Bielza et al., 2009; Castaneda et al., 2011). Reasons for not observing any fitness cost related with resistance could be as follows: 1) Resistance costs would be apparent only in specific environmental conditions, and thus these resistance costs would not be detected in experimental laboratory conditions (Foster et al., 2003; Bourguet et al., 2004). For example, Foster et al. (2003) showed that houseflies expressing knockdown kdr mutation, which grants resistance against pyrethroids and DDT, exhibit behavioral differences related with fitness in comparison with susceptible individuals. 2) Some pleiotropic effects might not be detected with current methods (inefficiency of the statistical or experimental method to detect costs) (Fry 1993). 3) There would not be any fitness cost related to insecticide resistance, or some modifiers would compensate for resistance costs (Coustau et al. 2000).

Table 4. Female survival values (in days) for all strains. Medians that share the same letter do not differ significantly in pairwise log-rank test (P < 0.01).

	Median	Mean	Std. dev.	Total N
GS	27[a]	22.40	11.47	50
GFR	26[a]	22.68	9.43	50
GFM-1	16[b]	17.36	8.38	50
GFM-2	19[c]	19.60	6.82	50

Table 5. Male survival values (in days) for all strains. Medians that share the same letter do not differ significantly in pairwise log-rank test (P < 0.01).

	Median	Mean	Std. dev.	Total N
GS	5[a]	10.42	7.68	50
GFR	17[b]	16.76	6.32	50
GFM-1	13[c]	13.84	4.95	50
GFM-2	13[c]	12.68	3.43	50

Although we did not observe any significant difference between resistant GS and susceptible GFR strains, after the first susceptible migration a significant reduction in fecundity and fertility was detected in the GFM-1 strain. The GFM-1 strain had significantly lower fecundity compared to all other strains, including the GFM-2 strain (Table 3). The GFM-2 strain had lower fertility compared to the GS and GFR strains, but they were not significantly different from the GFM-2 strain. However, it can be seen from Table 3 that the standard error of fertility is relatively high compared to the mean in the GFM-1 strain; this means that sample points are scattered far from the mean and confidence limits overlap with the GFM-2 strain. In addition, we also detected the expression of a fitness cost in female survival in the GFM-1 strain (Table 4), as GFM-1 females had the lowest survival. Male survival, on the other hand, showed a different pattern. Male survival of the susceptible GS strain was lowest among all strains, with the resistant GFR strain having the highest male survival (Table 5). However, female survival is a much more convenient parameter for fitness, because houseflies start copulating 12 h after emergence from pupa, and for females, usually a single copulation is sufficient for lifetime oviposition. This reduction of fitness parameters after the first migration is an interesting result and it is probable that it would have been caused by some side effects related to artificial selection, or incompatibility caused by differential selection between populations. For organophosphates, fitness of hybrid generations of resistant and susceptible populations was investigated for *Tribolium castaneum* by Haubruge and Arnaud (2001), who reported that fitness was independent from insecticide resistance genotype, and for *Culex quinquefasciatus* by El-Khatib and Georghiou (1985), whose study showed that fitness costs resulting from selection against temephos would be improved by hybridization. Roush and Plapp (1982) observed a decrease in biotic potential due to GST-based organophosphate resistance in *M. domestica*, but they did not observe any disadvantage of fitness in heterozygotes.

Selection with insecticides in treated areas and constant migration from untreated regions could be thought of as a source-sink model where continuous susceptible migration could significantly delay the development of resistance in treated regions, and combined with the assumed fitness costs of resistance this could hinder or even prevent evolution of resistance even more efficiently, as shown by many management models and field studies (Argentine et al., 1994; Georghiou, 1994; Raymond and Marguine, 1994; Peck, 1997; Lenormand and Raymond, 1998; Lenormand, 2002; Tyutyunov et al., 2008). In this study, our results showed that in a resistant laboratory strain of *Musca domestica* with continuous susceptible migration, both the level of resistance and the load of fitness costs could be eroded efficiently. An additional interesting finding is the reduction of some fitness parameters after the first generation of migration. We think that investigating the changes in fitness after migration is important; more detailed studies in other species with varying migration rates and generations would provide more information on this subject.

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