Chromosome contribution to malathion resistance in natural populations of *Drosophila melanogaster* collected from Turkey.

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Abstract

Previous studies showed that the malathion resistance in *Drosophila melanogaster* populations was under control of several genes that were located on the first, second, and third chromosomes. In order to describe the chromosomal localisation of malathion resistance developed in natural populations of *Drosophila melanogaster*, the contributions of genes were investigated using marker populations. As a result, the contribution of the second chromosome was found higher than the third chromosome. The contribution of the X-chromosome was not significant.

Key-words: *Drosophila melanogaster*, insecticide, resistance, malathion, localisation

Introduction

It is known that for a long period (approximately 20 years) *Drosophila melanogaster* populations have been treated with insecticides, especially with organophosphate malathion, for the pest management in agricultural fields. One of the major problems arising from the intensive applications of insecticides is the development of resistance. Nowadays, the synthetic insecticides have taken the place of organophosphate insecticides. However, insect populations rapidly develop resistance due to the intensive applications (Casida and Quistad, 1998). Consequently, development of resistance still remains a significant problem for pest control in agriculture. The aim of the present study is to determine the genes involved in the insecticide resistance in natural populations of *D. melanogaster* sampled from Turkey. To understand and determine the genetic basis of insecticide resistance, it is necessary to locate the genes responsible for insecticide resistance. For this purpose, the natural populations of the fruit fly *D. melanogaster* were used as a model organism.

Since 1952 the organophosphate insecticide malathion usage has risen in agriculture, because of its low mammalian toxicity (Casida and Quistad, 1998). For this purpose, malathion was preferred in this study. The researchers have reported that insecticide resistance in *D. melanogaster* has showed polygenic inheritance in laboratory populations (King and Somme, 1958; Singh and Morton, 1981; Halpern and Morton, 1987; Houpt *et al*., 1988). They reported that the resistance was observed on the second and the third chromosomes of *Drosophila*. On the contrary, Roush and McKenzie (1987) reported that monogenic inheritance of resistance is more common in natural populations of insects. To locate the resistance genes, genetic analysis was conducted using visible genetic markers and balancer chromosomes in *D. melanogaster* (King and Sømme, 1958; Hartl and Clark, 1997).

Material and Methods

a) The organism and environmental conditions

In this study, the natural populations of *D. melanogaster* were sampled from Antakya-Centre and Antalya-Serik locations of Turkey. The *Drosophila* marker stocks were provided from Bloomington *Drosophila* Stock Center. The marker stocks were *brown-scarlet* (*bw//bw;st//st*) and *Curly-Plum, Serrate-Stubble* (*Cy//Pm;Ser//Sb*). The flies were kept in a *Drosophila* culture room at 25±1ºC, relative humidity of 50-60%, and in 12 h light, 12 h dark periods on a standard *Drosophila* medium described by Bozcuk (Bozcuk, 1978).

b) Bioassays

To evaluate the susceptibility of each natural population, the filter-paper-contact method (Miyo *et al*., 2001) was used in all resistance measures. The virgin flies were collected and 3-5 days olds were used in bioassays. The filter papers (1 × 7.5 cm) and empty tubes (2.5 × 7.5 cm) were used in the experiments. The technical grade malathion insecticide (purity 98%) was provided from University of Groningen, Holland. Acetone (purity 99%) was used to dilute malathion. To obtain LC_{50} values, five concentrations of malathion with three replicates were used for each bioassay depending on the availability of flies. After 24 hours, the dead flies were counted. For each population, bioassays were conducted two times. As a result, the most resistant population to malathion was determined as Antakya-Centre, and the most sensitive population was determined as Antalya-Serik.

c) Determination of the chromosome contribution to resistance

To understand the chromosome contribution depending on X-chromosome to the malathion resistance, Antakya population (the most resistant natural population) was crossed with Antalya-Serik population (the most susceptible natural population). To examine the dominant and recessive factors affecting malathion resistance, Antakya population was crossed both with dominant and recessive marker stocks.

Results

a) The results of the bioassays and sex-linkage and the dominance of the factors

The experiments were replicated two times and five insecticide doses. To obtain LC_{50} values, five concentrations of malathion with three replicates were used for each bioassay. It is found that the most resistant population was Antakya, and most susceptible was Antalya-Serik populations and their reciprocals. The offspring (F_1) of these crosses were collected and tested their lethal concentrations of malathion. Sex-linkage was investigated by determining the differences in lethal concentrations between the sexes among the offspring from the crosses of Antakya females with Antalya-Serik males (cross Antakya × Serik), and the reciprocal cross Serik females with Antakya males (Serik × Antakya). The males from the Antakya × Serik cross received their X chromosome from their resistant mothers (Antakya females). The males from Serik × Antakya cross received their X chromosome from their susceptible mothers (Serik females). The results of the crosses and reciprocal crosses of Serik × Antakya determined that the resistance is between that of the parental

stocks (Table 1). Thus, it is clear that the resistance factors are not entirely dominant or entirely recessive.

Table 1. Lethal concentration (LC_{50}) values of malathion bioassays (mg/cm^2).

Strain	Sex	LC_{50} (95%CL)	Slope(± SE)	X^2
Antakya	F	3.850 (3.189-6.286)	3.118 ± 0.926	3.311
	M	2.458 (1.958-2.763)	10.936 ± 3.232	1.469
Serik	F	1.050 (0.215-1.423)	4.598 ± 1.644	1.318
	M	0.704 (0.00-0.00)	4.620 ± 3.55	0.828
Serik-Antakya Cross	F	1.820 (1.477-2.019)	4.883 ± 1.437	1.333
	M	1.631 (1.464-1.745)	8.191 ± 1.346	0.057
Antakya-Serik Cross	F	1.912 (1.721-2.063)	5.652 ± 0.709	2.513
	M	1.203 (0.838-1.442)	4.028 ± 0.743	3.324

F: female, M: Male

b) Dominant autosomal factors

To determine the effects of the dominant resistance factors (*RR*) in the resistant population Antakya and the recessive eye-color marker strain *bw//bw;st//st* (*bw: brown* eyes, *st: scarlet* eyes) were used. *Brown* (2-104.5 location) is located on second and *scarlet* (3-44.0 location) is located on third chromosome. The combination of both markers in homozygous condition produces white eyes on phenotype. The malathion resistant males (*RR*) from the natural population Antakya were crossed to females of the susceptible marker strain *bw//bw;st//st*. F_1 males (*r//Pm;r//Sb*) were backcrossed to resistant Antakya females (*RR*). The four type F_2 flies were collected. The phenotypes and the genotypes of this cross were white eyes (*bw//bw;st//st),* brown eyes (*bw//bw;st//R*), scarlet eyes (*bw//R;st//st*) and wild type (*bw//R;st//R*). The F_2 flies from this crosses were collected in respect of their eye color and tested their lethal consentration of Malathion. At the end of the bioassay experiments, the three F_2 phenotypes *scarlet*, *brown,* and *white* eyed individuals do not survive at all. The LC_{50} of the wild type (*bw//R;st//R*) was determined 70% death at the dose 1.673 mg/cm^2. As a result, we conclude that the presence of the factors determining resistance in Antakya population cannot be entirely dominant; it seems to be mostly recessive.

c) Recessive autosomal factors

To determine the effects of the recessive resistance factors (*rr*) in the resistant population Antakya, the dominant marker strain *Cy//Pm;Ser//Sb* was used. *Curly* (2-6.1 location) and *Plum* are located on second chromosome. *Serrate* (3-92.5 location) and *Stubble* (3-58.2 location) are located on third chromosome. Resistant females *(rr)* from the natural population Antakya were crossed to males of *Cy//Pm;Ser//Sb*. F_1 males *(r//Pm;r//Sb)* were backcrossed to resistant Antakya females *(rr)*. The four type F_2 flies were collected. The phenotypes and the genotypes of this cross were *Plum Stubble* (*r//Pm;r//Sb*), *Plum* (*r//Pm;r//r*), *Stubble* (*r//r;Sb//r*) and *wild type* (*r//r;r//r*). The F_2 flies from this crosses were collected and tested their lethal consentration of Malathion (Table 2). The LC_{50} of the *Plum* (*r//Pm;r//r*) flies was found more susceptible (1.162 and 0.906 µg/cm^2) than that of

Stubble (*r//r;Sb//r*) flies (1.410 and 1.209). The most resistant stock were found as wild type (r//r;r//r). Its lethal concentration was 2.650 and 2.168 μg/cm^2. This is the closer value to the resistant Antakya strain (LC_{50} 3.850 and 2.458 μg/cm^2). At this point, the effect recessive autosomal factors seems to contribute mostly to malathion resistance.

Table 2. Lethal concentration (LC_{50}) values of malathion bioassays (mg/cm^2).

Strain	Sex	LC_{50} (95%CL)	Slope(± SE)	X^2
Plum Stubble *(Pm Sb)*	F	*		
	M	*		
Plum *(Pm r)*	F	1.162 (0.187-1.642)	2.440 ± 0.886	3.759
	M	0.906 (0.0-0.969)	6.273 ± 3.903	0.398
Stubble *(r Sb)*	F	1.410 (0.921-1.634)	6.920 ± 1.980	5.400
	M	1.209 (1.303-1.581)	4.720 ± 1.710	6.316
Wild type (w.t.) *(r r)*	F	2.650 (2.442-2.883)	6.702 ± 0.971	3.118
	M	2.168 (1.713-2.506)	3.918 ± 0.89	0.364

* Highly susceptible (LC_{50} can't be counted)

Conclusion

In order to determine the genetic basis of malathion resistance, genetic analyses were conducted using visible genetic markers and balancer chromosomes in *Drosophila melanogaster* (King and Sømme, 1958; Hartl and Clark, 1997). Several studies showed that the malathion resistance in *D. melanogaster* populations has a polygenic inheritance (Houpt *et al*., 1988; Halpern and Morton, 1987). The genes involved in the polygenic inheritance producing insecticide resistance in the *D. melanogaster* populations have been determined. They are located on chromosomes 2 and 3 of *D. melanogaster*. This seems that the insecticide resistance is related to the chromosome 2, which controls several cytochrome P 450 species and which is responsible from malathion resistance (Hallstrom and Blanck, 1985; Houpt *et al*., 1988). It is reported that acetylcholinesterase, located on the third chromosome (Fournier *et al*., 1989), glutathione-S-transferases, mapped both on the second and third chromosome (Morton, 1993), and glucose-6-phosphate dehydrogenase on the X-chromosome (Morton and Holwerda, 1985) are associated with malathion resistance. The researchers have reported that X chromosome had no significant effect on resistance to the three organophosphates, and also that the second and third chromosomes made a significant contribution to the organophosphate resistance (Geerts *et al*., 2003; Miyo *et al*., 2002). For the malathion insecticide, the factor on the third chromosome had a larger affect that on the second chromosome (Miyo *et al.,* 2002).

The results presented here lead to the conclusion that the second chromosome contributes the most to the malathion resistance. This result is correlated with the result of Geerts *et al.,* (2003). The contribution of the second chromosome was found higher than the third chromosome. The contribution of the X-chromosome was not significant. The third chromosome contributes slightly. Further mapping research on the resistant natural populations will consolidate these findings observed in the present study.

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Microchaetae density is not greatly influenced by the overexpression of *akt.*

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Introduction

The notum of *Drosophila melanogaster* develops from a field of neuronal precursor tissue with the number of individual bristles being closely correlated to the number of sensory neurons formed (Jan and Jan, 1994). The density of the microchaetae on the dorsal notum is sensitive to both canonical and non-canonical Notch signalling at different stages of development (Tata and Hartley, 1995; Ramain *et al.,* 2001). Our laboratory has recently reported upon the role of the Huntingtin interacting protein-1 (Hip1) in neurogenesis (Moores *et al.,* 2008); we have found that analysis of microchaetae density provides a sensitive assay with which to approach subtle aspects of cell signalling. Two recent reports have investigated the activity of the *akt* kinase in *Drosophila* models of Huntington disease (Liévens *et al.,* 2008; Branco *et al.,* 2008). As part of our investigations into *Drosophila* homologues of genes with links to Huntington disease, we have explored the consequences of the overexpression of *akt* upon microchaetae density.

Drosophila melanogaster Strains and Culture

The *y w; apGal4/CyO (y w; P{GawB}ap^{md544}/CyO)* and the *y w; pnrGal4/TM3,UASy^{+} Ser (y w; P{GawB}pnr^{md237}/TM3, P{UAS-y.C}MC2, Ser^{1})* (Calleja *et al.,* 1996) driver lines plus the *w; UASlacZ^{4-1-2}* (Brand and Perrimon, 1993) and the *w; UASGFP* (Yeh *et al*., 1995) expression control lines were obtained from the Bloomington Drosophila Stock Center. The *w; UASAkt $^{1.1}$/CyO* line (*UASakt*) was described in Staveley *et al.* (1998). Males carrying the *UASlacZ*, *UASGFP* and *UASakt* responsive genes were each crossed to *apGal4* and *pnrGal4* virgin females and raised upon standard cornmeal-yeast-molasses-agar medium at 25ºC. Critical class males and females were