



## Occurrence of *P* element in natural populations of *Drosophila melanogaster* in Ukraine.

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### Abstract

Using PCR and sequencing we have demonstrated the presence of *P* elements in genomes of *Drosophila melanogaster* from natural populations of Ukraine. The degree of gonadal reduction revealed indirectly indicates low or, in some cases, zero activity of the mobile element in the inspected natural populations of *Drosophila*. All studied populations have been found to represent the M' cytotype. In this study, we have shown for the first time that *P* element, absent two decades ago, has invaded Ukrainian populations of *Drosophila melanogaster*. This invasion is part of the worldwide expansion of *P* element in *Drosophila melanogaster*. In Ukraine, an initial stage of the invasion has been suggested to occur, and further evolution of the M' cytotype into P cytotype is expected.

Keywords: *P* element, *Drosophila melanogaster*

### Introduction

Recent data suggest that according to different estimates up to 22 percent of the *Drosophila melanogaster* genome is represented by mobile elements (ME) pertaining to diverse families (Kapitonov and Jurka, 2003). The *P* element family (autonomous ME, 2.9kb long) includes MEs capable of promoting so-called P-M hybrid dysgenesis under a certain crossing scheme (Kidwell, 1985). Hybrid dysgenesis is usually accompanied by such effects as high gonadal reduction frequency, elevated rates of mutation, and recombination in males (Broadhead *et al.*, 1977). Based on the presence of *P* element and the hybrid dysgenesis manifestation all known fruit fly lineages are divided into three major types: a) no *P* element (M cytotype), b) *P* element present (P and Q cytotypes) (Kidwell, 1985). Beside these three, there are also two variants: M' (the M type yet containing *P* element) and P', the P strain unable to repress the effects of hybrid dysgenesis. Hybrid dysgenesis usually manifests itself in reduction of the size and stage of development of gonads in *Drosophila* of the F1 generation from crosses between *P*-element-containing males and females lacking *P* element, and is the result of *P* element movement in the fly germ cells. Although the exact nature of the process of *P* element movement suppression (as a defensive mechanism against GD) is still poorly understood, the two current hypotheses (that are not mutually exclusive) link it either to the activity of suppressor proteins produced by incomplete copies of *P* elements (Rio, 1990) or the activity of Piwi-interacting (pi) RNAs (Simmons *et al.*, 2007; Jensen *et al.*, 2008). Whatever the mechanism, flies inherit maternally a means to suppress movement of *P* element, and this is the basis of their division into so-called cytotypes. Present day cytotypes are distinguished depending on the ability of flies to induce (tested on males) or repress (tested on females) active movement of *P* elements. In this way, P cytotype lineages have both inducing and repressing abilities, P' cytotype strains have only inducing ability, Q strains have only repressing ability, and M cytotype flies have

neither (see in Itoh *et al.*, 2007) and are devoid of *P* elements. *M'* cytotype defines flies that lack both inducing and repressing abilities, but still contain *P* elements (Bingman *et al.*, 1982).

In laboratory lineages and natural populations of *Drosophila* collected before 1950, *P* element has not been detected (Kidwell, 1983). Its invasion of the *Drosophila melanogaster* genome is believed to have started somewhere in the middle of the twentieth century in the Caribbean or southeastern North America, probably originating through a horizontal transfer from *D. willistoni* (Daniels *et al.*, 1990; Clark and Kidwell, 1997; Itoh *et al.*, 2007). By the 1980s, the invasion had spread all over the world including Europe, but it hadn't been found to occur in populations from the former Soviet Union and Australia (Kidwell, 1983; Zakharov, 1984). In the 1990s, it was detected in Japanese populations (Gamo *et al.*, 1990). Concerning Ukraine, a work published in 2006 demonstrated the presence of incomplete copies of this element in a natural population from Uman collected in 1983 (Kovalenko *et al.*, 2006). However, no detailed research on ME in natural populations of *Drosophila* in Ukraine has been carried out so far.

The aim of the present study was to investigate the occurrence of *P* element in natural populations of *Drosophila melanogaster* in Ukraine.



Figure 1. Fly collection sites.

## Materials and Methods

We studied lineages of *Drosophila melanogaster* originating from different natural populations collected at several locations in Ukraine in 2008 and 2009 (Figure 1), as well as the

laboratory line *Canton S*, a wild type lineage that may sometimes not contain the mobile *P* element (Roberts, 1986), being part of the collection of General and Molecular Genetics Department of Taras Shevchenko National University of Kyiv and courteously granted by Genetics Department of Lomonosov Moscow State University in 1992.

Cytotype determination was based on the gonadal dysgenesis (GD) assay and included two kinds of crosses (Kidwell *et al.*, 1977; Engels and Preston, 1980) – female *Canton-S* (M cytotype) were crossed with wild caught males to test the GD induction potential in wild flies (cross A), and wild females were crossed with Harwich (P cytotype) males to test the repression potential of the tested wild flies (cross B). Cytotype was determined as described in Itoh *et al.* (2001). Gonad biotomy and visual inspection of their developmental status was used to measure GD. Only unilateral and bilateral ovary and seminal gland reduction were counted. For each population, 50 individuals of each sex were analyzed. Percent ratio of GD was calculated using the formula  $\%GD = \frac{1}{2}\%GD(1) + \%GD(2)$ , where  $\%GD(1)$  stands for the proportion of individuals with unilateral reduction of the ovary/seminal gland taken as a percent of the whole sample;  $\%GD(2)$  means the proportion of individuals with bilateral gonadal reduction taken as a percent of the whole sample.

We used a 437 bp region of *P* element DNA sequence to look for possible base substitutions and confirm the results of *P* element detection by PCR. This region is believed to be part of all known types of incomplete *P* element copies and the complete one. Total DNA was extracted from adult individuals of each population using QIAamp DNA Micro Kit (Qiagen, USA). We employed PCR to amplify the 437 bp *P* element fragment using primers 5'-ACGTTTGCTTGTTGAGAGGA-3' and 5'-AACAGGACCTAACGCACAGT-3' specific to the abovementioned fragment ranging from the 41th to the 477th base of the gene coding for the *P* element transposase.

Sequence alignment was performed using the Vector NTI software.

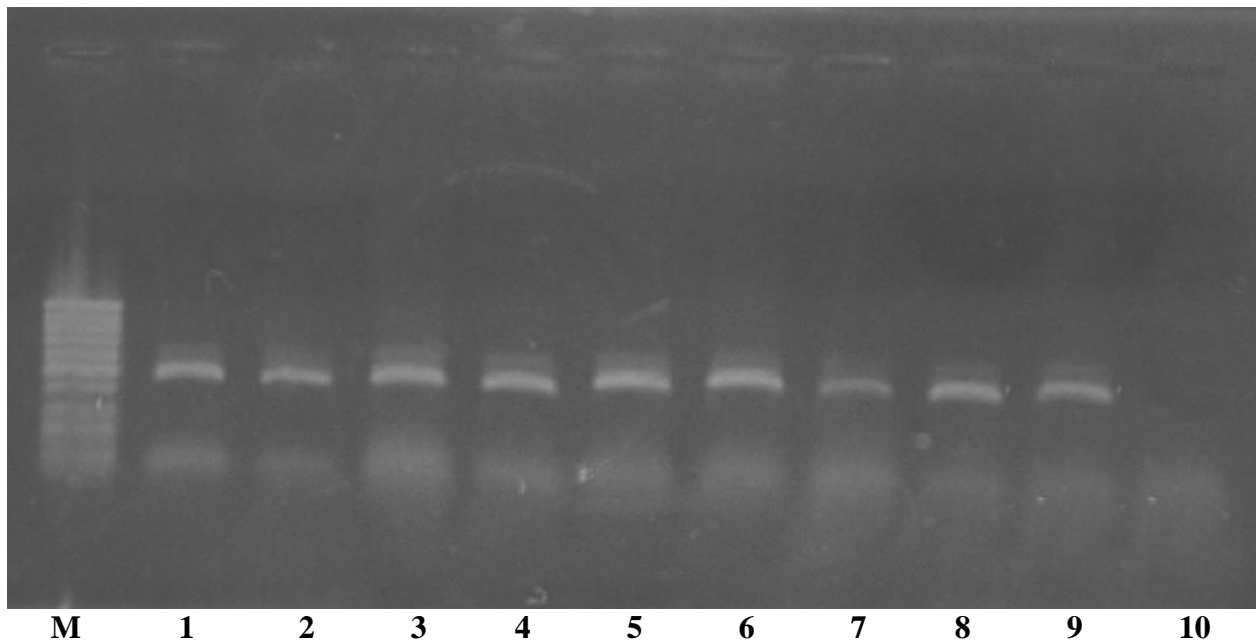


Figure 2. PCR analysis of fly lineages. M – molecular weight marker, 1-9 – *P* element transposase fragments in DNA of flies from the studied natural populations, 10 – DNA of the *Canton-S* line (no fragments).

## Results and Discussion

Individuals of *Drosophila melanogaster* were collected in different regions of Ukraine planned so as to form a latitudinal cross section of the country from the north to the south, as up to the 1980s not all natural populations of this species contained *P* elements, and specifically populations from the territory of the former Soviet Union had not been found to contain MEs of this type at all (Kidwell, 1985).

As it can be seen from Figure 2, DNA of all lineages, except for *Canton S*, produced products of the expected length. To further ascertain that these bands represented *P* elements, we extracted and sequenced the products. Figure 3 demonstrates the region of *P* element (reference: GenBank accession #AB331393) we amplified. The amplified sequences were identical to the reference, and we found no base substitutions in any of the studied populations. Therefore, we can state that all of the studied natural populations contain copies of *P* element. However, insomuch as the primers were specific to a small fragment of the transposase gene, we were unable to distinguish which copies were full-size and what types of incomplete copies occur. Size distribution of the detected copies requires further investigation.

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(41)      ACGTTTGCTT GTTGAGAGGA AAGGTTGTGT GCGGACGAAT TTTTTTTTGA      (90)
(91)      AAACATTAAC CCTTACGTGG AATAAAAAAA AATGAAATAT TGCAAATTTT      (140)
(141)     GCTGCAAAGC TGTGACTGGA GTAAAAATTAA TTCACGTGCC GAAGTGTGCT      (190)
(191)     ATTAAGAGAA AATTGTGGGA GCAGAGCCTT GGGTGCAGCC TTGGTGAAAA      (240)
(241)     CTCCCAAATT TGTGATACCC ACTTTAATGA TTCGCAGTGG AAGGCTGCAC      (290)
(291)     CTGCAAAAAGG TCAGACATTT AAAAAGGAGGC GACTCAAACGC AGATGCCCGTA      (340)
(341)     CCTAGTAAAG TGATAGAGCC TGAACCAGAA AAGATAAAAAG AAGGCTATAC      (390)
(391)     CAGTGGGAGT ACACAAACAG AGTAAAGTTTG AATAGTAAAA AAAATCATTT      (440)
(441)     ATGTAAACAA TAACGTGACT GTGCGTTAGG TCCTGTT      (477)

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Figure 3. The 437 bp region of the *P* element of *Drosophila melanogaster* we amplified.

Occurrence of *P* element in all studied populations suggests that this transposon may have spread all over the territory of Ukraine since the 1980s.

Gonadal dysgenesis and cytotype determination results are summarized in Table 1. As seen from Table 1, both the GD induction (cross A) and GD repression (cross B) potentials of all studied populations are low. This indicates that all populations represent the M type cytotype. The presence of *P* elements demonstrated by PCR indicates that they all have specifically M' cytotype, *i.e.* they have yet acquired *P* element but still lack the accompanying molecular machinery which evolves during further stages of *P* element invasions. The results of GD induction assessment crosses (cross A) suggest that the *P* elements are still not actively transposing in these populations. Although we didn't study the exact size distribution of different *P* element copies, the absence of notable *P* element transposition in the populations studied may also indirectly suggest that they lack complete copies. The logic behind this inference is that complete copies are the only ones capable of active transposition, and other types of *P* elements are able to move only in the presence of complete copies which serve as a transposase donor. However, this requires further investigation.

It is known from literature that natural populations of *Drosophila melanogaster* are characterized with a phenomenon called "mutation outburst" (Golubovski, 1985) detected as an enhanced rate of mutation compared to the background level. There are suggestions that the activity of mobile elements may account for such outbursts (Golubovski, 1985). Our previous research didn't

find any signs of such events in natural populations of *Drosophila* in 2005-2006 (Protsenko and Kozeretska, 2006, 2007). Therefore, the genomes of the flies from the populations inspected do bear *P* element, but its active movement was not registered at the time of the study.

Table 1. GD and cytotype determination results.

Fly lineage	GD% in F1 offspring						Cytotype
	unilateral GD		bilateral GD		Total GD		
	cross A	cross B	cross A	cross B	cross A	cross B	
Conton-S	0	9	0	60	0	69	M
Odesa	1	14	0	68	1	82	M'
Cooling Pond	0	18	0	48	0	66	M'
Magarach	0	3	0	88	0	91	M'
Uman	1	10	0	62.5	1	72.5	M'
Kyiv	0	4	2	2	2	26	M'
Lubny	0	7	0	18	0	25	M'
Pryatyn	0	2	0	60	0	62	M'
Varva	0	9	0	76	0	85	M'
Chornobyl	0	11	0	62	0	73	M'
Harwich		0		0		0	

Therefore, the presence of *P* element copies has been registered in all studied populations of *Drosophila melanogaster* in Ukraine. All populations in the study represented M' cytotype, which suggests a comparatively recent invasion of Ukrainian populations of *Drosophila melanogaster* by this element. Ukrainian populations, thus, are experiencing a transitional state of a *P* element invasion, and evolution of P cytotype is expected in future. Search for possible occurrence of P cytotype in other regions of Ukraine not investigated in this study would potentially bring insights into the timing of this transitional period, as in case P cytotype is found somewhere, its sooner spread over the territory of Ukraine may be expected. The low level of gonadal reduction in A crosses suggests the absence of active transposition of mobile elements in natural populations of *Drosophila* in Ukraine. The results obtained are interesting not only on a local scale. They contribute to the worldwide pattern of *P* element invasion of *Drosophila melanogaster* populations.

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### **Effects of fumonisin B<sub>1</sub> to developmental stages of F<sub>2</sub> offspring of *Drosophila melanogaster*.**

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## **Introduction**

Mycotoxins are secondary metabolites of moulds, and their compounds have toxic effects on the living organisms. The toxic effect of mycotoxins on animal and human health is referred to as mycotoxicosis (Peraica *et al.*, 1999). Fumonisin B<sub>1</sub> is a mycotoxin produced by mainly in *Fusarium moniliforme*. Fumonisin (FBs) family includes fumonisin A<sub>1</sub>, A<sub>2</sub>, B<sub>1</sub> and B<sub>2</sub>. Fumonisin B<sub>1</sub> is the best known and worked on (Gelderblom *et al.*, 1988; Bezuidenhout *et al.*, 1988). Fumonisin occur infrequently in foodstuffs, such as sorghum, asparagus, rice, beer, and mung beans (Creppy, 2002).

It has been shown that Fumonisin B<sub>1</sub> causes a number of heavy damage effects in animals, including equine leukoencephalomalacia (Gelderblom *et al.*, 1988; Marasas *et al.*, 1988; Kellerman *et al.*, 1990), porcine pulmonary edema (Harrison *et al.*, 1990), hepatotoxicity (Gelderblom *et al.*, 1996), nephrotoxicity, and genotoxicity (Knasmuller *et al.*, 1997).

Since Fumonisin B<sub>1</sub> has a toxic effect on many organisms, we have investigated any lasting impact on development stages of *Drosophila melanogaster*. *Drosophila melanogaster* provides a powerful system in which to use genetic and molecular approaches to investigate human genetic diseases (Chien *et al.*, 2002). It also has many advantages for a model organism, because, known as fruit fly, *Drosophila melanogaster* has several features, such as different ecological adaptation to the environment, transport giant chromosomes, simple food requirements, and having a lot of genetic variation.

## **Materials and Methods**

Oregon R wild type strain of *Drosophila melanogaster* was used in this work. All experiments were carried out 25 ± 1°C and at approximately 60% of relative humidity. The fly stocks and experimental groups were kept on Standard *Drosophila* Medium (SDM) containing maize-flour, agar, sucrose, dried yeast and propionic acid (Çakır and Bozcuk, 2000). Fumonisin B<sub>1</sub> was dissolved 10% DMSO (Dimethyl sulfoxide). 1, 3, 5 and 10 µM test solutions were prepared and added in 50 ml bottles of SDM. For experiments average same aged individuals obtained from stock culture and for