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P-M STATUS OF *DROSOPHILA MELANOGASTER* NATURAL POPULATIONS IN UKRAINE

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P element has invaded Drosophila melanogaster populations all over the world in less than 50 years. Our previous studies indicate that this transposon is present in Ukrainian populations as well. Drosophila react to P element invasion by developing a special defensive cellular state called P cytotype, as opposed to their natural M cytotype which cannot defend the flies against the deteriorating effect of P element activity in their genomes. Previous studies have shown that Ukrainian populations have not developed P cytotype, suggesting a recent invasion by P element. In the present study, we analyze the cytotype status of Ukrainian populations of Drosophila melanogaster and demonstrate for the first time the existence of the so-called P' cytotype. This cytotype has been predicted theoretically, but has never been demonstrated in nature on the population level.

Keywords: Drosophila melanogaster, P element, cytotype, transposon, hybrid dysgenesis.

Introduction. According to various estimates, up to 22% of the *Drosophila melanogaster* genome is made up by mobile genetic elements (MEs) of diverse families [1]. The P element family consists of MEs that are known to promote deviant phenotypes, collectively called the *hybrid dysgenesis syndrome* (HD), as a result of a certain crossing scheme [2]. Hybrid dysgenesis usually manifests itself in a number of aberrant traits, such as increased frequency of undeveloped gonads, elevated mutation rates and recombination in males [3]. Based on the flies' ability to repress the syndrome, all known drosophila lineages are classified into several groups, the so-called cytotypes. Crosses *M females x tested males* are used to assess the male lineage's ability to induce HD in F1 progeny. The reverse cross *tested female x P male* are used to assess the female lineage's ability to repress HD in F1 progeny. The basic natural state when P element is absent from the genome is called the *M cytotype*. Flies with M cytotype are neither able to repress nor induce HD in F1 in any crosses. *M' cytotype* is basicaly the same in regard to HD repression but describes genomes where P element (mostly inactive) is present. The *Q cytotype* gives flies the ability to repress HD, but such flies cannot induce HD if crossed with M lineages. The *P cytotype* is the most advanced cellular state with notable ability to repress HD, as well as induce it if crossed with M lineages [2, 4, 5]. There is still another cytotype, called *P' cytotype* (no HD repression, but HD induction ability present), which has been

predicted theoretically [6], but has never been observed in nature on the population level. The most prominent trait in dysgenic F1 flies (M female X P male) is gonadal dysgenesis (GD). Females are more susceptible to GD and demonstrate small undeveloped ovaries without eggs inside; in many case the ovaries may be even totally absent.

Albeit the exact nature of GD repression is not clearly understood so far, three mechanisms have been proposed to limit the transposition of P element. These are a) production of repressor proteins by internally deleted P elements, b) the activity of Piwi-interacting RNAs, or piRNAs [7, 8], and RNA editing [9].

P element has not been detected in laboratory strains established from flies collected before 1950 [10]. Several lines of experimental evidence clearly indicate that P element has been horizontally transferred to *D. melanogaster* from another species, *D. willistoni*, somewhere in the 1950s in North America [4, 11, 12]. Just a three decades later, P element had been found all around the globe, except for Australia and the Soviet Union [10, 13, 14].

First report of P element in natural populations of *D. melanogaster* from Ukraine (Uzhhorod, Uman, Yalta, Zaporizhzhya, and Chornobyl) dates back to the mid-1980s [15, 16]. All populations, however, demonstrated M' cytotype in GD assays. Later, P element presence was confirmed in Uman populations [17]. In our previous study [18], we have confirmed P element presence in all the previously reported populations and found it to be spread apparently all over Ukraine. However, detailed studies of P element-related cytotype development in natural populations of *D. melanogaster* in Ukraine so far are lacking. Also, which specific types of P element exist in Ukrainian populations remains a question.

The aim of the present study was to investigate the P-M status of Ukrainian populations of *D. melanogaster*.

Materials and methods

We studied isofemale lines [19] set up with flies collected from eight natural populations (Kyiv, Uman, Varva, Odesa, Magarach, and three populations from the Chornobyl zone of alienation, i.e. the city of Chornobyl, Cooling Pond [Chonobyl NPP], and Poliske) distributed so that they represented a latitudinal cross of Ukraine (Figure 1).

Fig. 1. Map of the study areas where *D. melanogaster* populations were sampled

We used laboratory strains *Canton-S* and *Harwich* as standards for M and P cytotypes, respectively, to generate dysgenic crosses with wild-derived flies [20].

Cytotype was determined based on the gonadal dysgenesis assay [21, 22] involving two kinds of crosses. GD induction potential was assessed based on crosses between female *Canton-S* and wild-caught males, and GD repression potential was assessed based on GD proportion in the crosses between wild-caught females and *Harwich* males. Cytotype was determined as described in [23]. GD was measured based on gonadal biotomy and visual inspection of the developmental status of gonads. We

counted unilateral and bilateral reduction of female gonads. Percent ratio of GD was counted as %GD = S%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.

DNA was extracted from 25 to 30 individuals using the QIAmp DNA Micro Kit (Qiagen, USA).

P element was detected by PCR with the following primers: 5'-ACGTTTGCTTGTTGA GAGGA-3' and 5'-AACAGGACCTAACGCA CAGT-3'. We amplified a 437 bp region of P element (41-477) which is universally present in all known P elements [8]. The PCR profile was as follows: denaturation 95°C/4 min; 30 cycles: denaturation 95°C/40 s, annealing 58°C/40 s, elongation 73°C/40 s; final elongation 71°C/10 min.

PCR products were visualized in 1% agarose gel electrophoresis and extracted from gel using QIAquick PCR Purification Kit (Qiagen, USA) for sequencing. Amplicons were sequenced in the Engencore laboratory of the University of South Carolina (USA). The obtained sequences were aligned in VectorNTI (Invitrogen, USA) and verified against a reference sequence (GenBank ID: AB331393).

Results and discussion

Before the GD assay, we performed diagnostic PCRs to detect P element in the studied populations. This detection was done to ensure that all populations for which cytotype was to be determined contained P element fragments in their genomes. The results of the PCRs are shown in Figure 2.

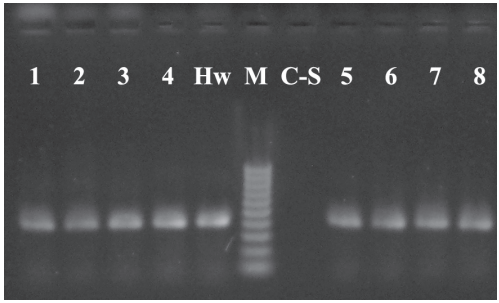

Fig. 2. PCR of P element in wild caught flies before GD assay: *Hw* – strain *Harwich* (serving as positive control), *C-S* – strain *Canton-S* (negative control), M – 100 bp molecular weight marker. Populations: 1 – Poliske, 2 – Cooling Pond, 3 – Chornobyl, 4 – Kyiv, 5 – Varva, 6 – Uman, 7 – Odesa, 8 – Magarach

Summary of the reciprocal crosses and GD percent for each population is shown in Table 1. Three out of the eight studied populations demonstrate M' cytotype, meaning that these populations have been invaded by P element (as also evident from Figure 2) but have not developed cellular defensive mechanisms against GD. The rest of the populations clearly demonstrate cytotype P', indicating the P element is active in their genomes and can induce GD in crosses with M-females. However, these P' populations still lack the ability to repress the activity of P element, just as do the M' populations.

Our study corroborates for the first time the presence of the theoretically predicted P' cytotype in natural populations of *D. melanogaster*. It also demonstrates that the cytotype of Ukrainian populations has evolved for the last few decades. Although some of the populations were studied for the first time, others (Uman, Chornobyl, and Magarach) have been inspected in the mid-1980s [15, 16] and demonstrate a transition from M' to P' cytotype.

Our data open an avenue for further research, as the existence of P' cytotype, in Ukrainian populations in particular and in fruit flies in whole, raises a number of

Table 1. Summary of the GD assay results**.** The cross *Canton-S* X *Harwich* served as a control and demonstrates that *Canton-S* has no GD repression potential and *Harwich* does have a strong GD induction potential, corroborating thus that the control fly lineages used were appropriate for GD assay (also note the absence of *P* element in *Canton-S* shown in Figure 2)

Crosses, ♀ x ♂	GD incidence in F1				%GD	Cytotype
	absent	unilateral	bilateral	total		
Poliske X *Harwich*	16	39	177	232	84.6	P’
Canton-S X Poliske	164	24	109	297	40.7	
Chornobyl X *Harwich*	34	24	257	315	85.4	P’
Canton-S X Chornobyl	270	37	169	476	39.4	
Cooling Pond X *Harwich*	122	123	366	611	70	P’
Canton-S X Cooling Pond	426	24	37	487	10.1	
Kyiv X *Harwich*	32	20	227	279	84.9	P’
Canton-S X Kyiv	143	20	43	206	25.7	
Uman X *Harwich*	48	40	313	401	83	P’
Canton-S X Uman	139	52	154	345	52.2	
Varva X *Harwich*	9	41	233	283	89.6	M’
Canton-S X Varva	434	17	17	468	5.4	
Odesa X *Harwich*	10	5	103	118	89.4	M’
Canton-S X Odesa	104	2	0	106	0.9	
Magarach X *Harwich*	6	13	72	91	86.3	M’
Canton-S X Magarach	246	7	19	272	8.3	
Total				**4987**		
Canton-S X *Harwich*	0	0	65	65	100	OK

questions. First of all, we don’t know how long this cytotype has existed in the studied populations and whether it is transitive or stable. Most probably this specific cytotype is not stable and is an intermediate stage in the evolution of P cytotype from M. The reason is that P’ cytotype does not have GD repressing potential, while the evolution of cytotypes is generally believed to specifically aim at developing defensive mechanisms against an active P element. Although we did not analyze what kinds of P elements were present in the studied populations, the existence of P’ cytotype by definition requires active full-size P elements capable of expressing P transposase, because GD, just as HD in whole, primarily results from P element transposition.

Also, it remains unclear which of the two detected cytotypes preceded the other in Ukrainian populations. The point is that both M’ and P’ cytotypes lack P element repression potential. However, a clear difference between the two is that M’ cytotype also lacks GD induction potential. This means that, most probably, these M’ populations lack active P elements. Following general logic, inactive (internally deleted) P elements evolve from active full-size elements via internal deletions, but not vice versa. This suggests that either P’ cytotype preceded the M’ cytotype in Ukrainian populations or there have been at least two separate invasions, one by incomplete (inactive) P element copies and the other by active elements. From literature, M’ cytotype was detected in

Ukrainian populations long before P'. On the other hand, the number of populations studied in the mid-1980s was very limited, and these studies may have omitted populations or groups of individuals possessing P' cytotype.

Besides, although GD manifestation generally requires rearing temperatures above 27°C [2], the absence of P element repression potential in P' cytotype brings up the question of how much GD occurs naturally in the hosting populations and whether there is any correlation between GD in P' populations and the average temperatures in August when the flies reproduce most actively. The existence of such a correlation would impose additional constrains on P' cytotype endurance and spread in natural populations. As data on P' cytotype geographic distribution is lacking, it is also interesting if this cytotype is possible in warmer climates where temperatures above 27°C are more common during the reproductive season. From Table 1 with reference to the map in Figure 1, P' populations in our study appear to concentrate in the Chornobyl zone and protrude to Kyiv and Uman, which is in the northern to central parts of Ukraine. Both populations inhabiting warmer climates within Ukraine (Odesa and Magarach) show no signs of P' cytotype and possess M' cytotype. Unfortunately, the number of populations we studied is too small to draw strong conclusions on this putative geographic pattern and more detailed and thorough studies are needed.

Admittedly, the nature of P' cytotype requires and deserves further research, as it might potentially bring important insights into the population biology of cytotypes, an area hardly touch in scientific literature.

Conclusions

Our study demonstrates for the first time the presence of the theoretically predicted P' cytotype in natural populations of *Drosophila melanogaster* on the population level. The presence of this cytotype indicates that the invasion of Ukrainian fruit fly populations by *P* elements has launched the evolution of cytotype. The existence of P' cytotype raises a number of questions about the mechanisms suppressing *P* element activity in flies with this cytotype. P' flies do not have the GD repression ability but still have active *P* elements in their genomes, so it's interesting what factors suppress the activity of this transposon. As GD requires rearing temperatures above 27°C, we hypothesize that P' populations must be limited to temperate climates. Among the populations we studied, none of the two southernmost populations showed P' cytotype. However, corroboration of our hypothesis requires further research involving much more populations sampled from a broader geographic range.

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References

1. *Kapitonov V.V., Jurka J.* Molecular paleontology of transposable elements in the *Drosophila melanogaster* genome // Proc. Natl. Acad. Sci. USA. – 2003. – Vol. 100. – P. 6569–6574.
2. *Kidwell M.G.* Hybrid dysgenesis in *Drosophila melanogaster*: nature and inheritance of *P* element regulation // Genetics. – 1985. – Vol. 111. – P. 337–50.
3. *Broadhead R.S., Kidwell J.F., Kidwell M.G.* Variation of the recombination fraction in *Drosophila melanogaster* females // J. Hered. – 1977. – Vol. 68. – P. 323–326.
4. *Itoh M., Takeuchi N., Yamaguchi M., Yamamoto M.-T., Boussy I.A.* Prevalence of full-size *P* and *KP* elements in North American populations of

Drosophila melanogaster // Genetica. – 2007. – Vol. 131. – P. 21–28.
5. *Bingman P.M., Kidwell M.G., Rubin G.M.* The molecular basis of P-M hybrid dysgensis: the role of the *P* element, a P-strain-specific transposon family // Cell. – 1982. – Vol. 29. – P. 995–1004.
6. *Quesneville H., Anxolabehere D.* Dynamics of transposable elements in metapopulations: a model for P element invasion in *Drosophila* // Theoretical Population Biology. – 1998. – Vol. 54. – P. 175-193.
7. *Simmons M. J., Ryzek D.-F., Lamour C., Goodman J. W., Kummer N. E., Merriman P. J.* Cytotype regulation by telomeric *P* elements in *Drosophila melanogaster*: evidence for involvement of an RNA interference gene // Genetics. – 2007. – Vol. 176. – P. 1945–1955.
8. *Jensen P.A., Stuart J.R., Goodpaster M.P., Goodman J.W., Simmons M.J.* Cytotype regulation of *P* transposable elements in *Drosophila melanogaster*: repressor polypeptides or piRNAs? // Genetics. – 2008. – Vol. 179. – P. 1785–1793.
9. *Fukui T., Itoh M.* RNA editing in P transposable element read-through transcripts in *Drosophila melanogaster* // Genetica. – 2010. – Vol. 138. – P. 1119–1126.
10. *Kidwell M.G.* Evolution of hybrid dysgenesis determinants in *Drosophila melanogaster* // Proc. Natl. Acad. Sci. USA. – 1983. – Vol. 80. – P. 1655–1659.
11. *Daniels S.B., Preston K.P., Strausbaugh L.D., Kidwell M.G., Chovnik A.* Evidence for horizontal transmission of the *P* transposable element between *Drosophila* species // Genetics. – 1990. – Vol. 124. – P. 339–355.
12. *Clarck J.B., Kidwell M.G.* A phylogenetic perspective on *P* transposable element evolution in *Drosophila* // Proc. Natl. Acad. Sci. USA. – 1997. – Vol. 94. – P. 11428–11433.
13. *Zakharov I.K.* The role of P- and M-cytotypes in the control of mutation in unstable alleles of the gene *singed* in *Drosophila melanogaster* // Genetika. – 1984. – Vol. 20. – P. 42–50 (in Russian).
14. *Gamo S., Sakajo M., Ikeda K.* Temporal distribution of *P* elements in *Drosophila melanogaster* strains from natural population in Japan // Jpn. J. Genet. – 1990. – Vol. 65. – P. 277–285.
15. *Anxolabehere D., Nouaud D., Periquet G., Tchen P.* P-element distribution in Eurasian populations of *Drosophila melanogaster*: a genetic and molecular analysis // Proc. Natl. Acad. Sci. USA. – 1985. – Vol. 82. – P. 5418–5422.
16. *Ronsseray S., Lehmann M., Anxolabehere D.* Copy number and distribution of P and I mobile elements in *Drosophila melanogaster* populations // Chromosoma. – 1989. – Vol. 98. – P. 207–214.
17. *Kovalenko L.V., Zahkarenko L.V., Voloshyna M.A., Karamysheva T.V., Rubtsov N.B., Zakharov I.K.* The behavior of transposons *hobo* and *P* in the unstable line *yellow*$^{2\text{-}717}$ of *Drosophila melanogaster* and its offspring after crosses with a laboratory line // Genetika. – 2006. – Vol. 42. – P. 748–756 (in Russian).
18. *Rozhok A., Bilousov O., Protsenko O.V., Zhuk O., Kozeretska I.A.* Occurrence of P element in natural populations of *Drosophila melanogaster* in Ukraine // Dros. Inf. Serv. – 2009. – Vol. 92. – P. 73–78.
19. *David J.R., Gibert P., Legout H., Petavy G., Capy P., Moreteau B.* Isofemale lines in *Drosophila*: an empirical approach to quantitative trait analysis in natural populations // Heredity. – 2005. – Vol. 94. – P. 3–12.
20. *Roberts D.B. Drosophila*: a practical approach. – Oxford, 1986. – 295 pp.
21. *Kidwell M.G., Kidwell J.F., Sved J.A.* Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility and male recombination // Genetics. – 1977. – Vol. 86. – P. 813–833.
22. *Engels W.R., Preston C.R.* Components of hybrid dysgenesis in a wild population of *Drosophila melanogaster* // Genetics. – 1980. – Vol. 95. – P. 111–128.
23. *Itoh M., Sasai N., Inoue Y., Watada M. P* elements and P-M characteristics in natural populations of *Drosophila melanogaster* in the southernmost islands of Japan and in Taiwan // Heredity. – 2001. – Vol. 86. – P. 206–212.

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Р-М СТАТУС ПОПУЛЯЦІЙ *DROSOPHILA MELANOGASTER* В УКРАЇНІ

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Р-елемент проник у популяції *Drosophila melanogaster* по всьому світу за менш як 50 років. Наші попередні дослідження показують, що цей транспозон присутній також і в українських популяціях. У відповідь на появу Р-елемента в геномі *Drosophila* розвивають спеціальний захисний клітинний механізм, що називається цитотип Р, на відміну від природного цитотипу М, котрий не захищає від руйнівного впливу активності Р-елемента на геноми плодових мух. Згідно з результатами попередніх досліджень українські популяції ще не розвинули цитотип Р, що вказує на недавню появу Р-елемента. В даній роботі ми аналізуємо цитотипний статус українських популяцій *Drosophila melanogaster* та вперше показуємо існування в природі так званого цитотипу Р'. Цей цитотип був передбачений теоретично, але ніколи не був знайдений у природі на популяційному рівні.

Ключові слова: *Drosophila melanogaster*, Р-елемент, цитотип, транспозон, гібридний дисгенез.

Р-М СТАТУС ПОПУЛЯЦИЙ *DROSOPHILA MELANOGASTER* В УКРАИНЕ

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Р-элемент проник в популяции *Drosophila melanogaster* по всему миру за менее чем 50 лет. Наши предыдущие исследования показывают, что этот транспозон присутствует также и в украинских популяциях. В ответ на появление Р-элемента в геноме *Drosophila* развивают специальный защитный клеточный механизм, называемый цитотипом Р, в отличие от их естественного цитотипа М, который не защищает от разрушительного действия активности Р-элемента на геномы плодовых мух. Согласно результатам предыдущих исследований, украинские популяции пока что не развили цитотип Р, что указывает на недавнее появление Р-элемента. В данной работе мы анализируем цитотипный статус украинских популяций *Drosophila melanogaster* и впервые показываем существование в природе так называемого цитотипа Р'. Этот цитотип был ранее предсказан теоретически, но никогда не был показан в природе на популяционном уровне.

Ключевые слова: *Drosophila melanogaster*, Р-элемент, цитотип, транспозон, гибридный дисгенез.