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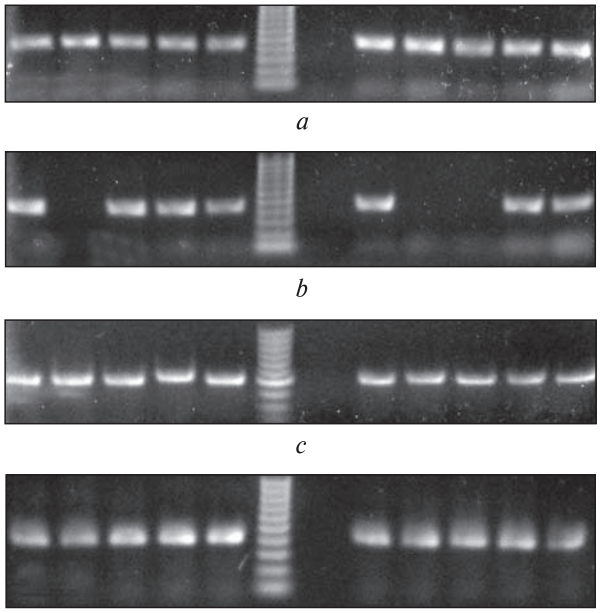
ON THE PERSISTENCE OF *P* ELEMENT IN CULTURED LINEAGES OF *DROSOPHILA MELANOGASTER*

P transposon is known to have invaded the Drosophila melanogaster genome in the 1950s as a result of horizontal transmission from D. willistoni. Part of the evidence supporting the timing of its invasion comes from analyses of cultured drosophila lineages originating from wild flies cultivated long time in laboratory before analysis. Such analyses have shown that P element was absent from the genomes of cultured lineages established from wild flies caught from the wild before the 1950s. Although the hypothesis of P element transmission has obtained multiple lines of evidence and is beyond doubt today, we decided to test whether analysis of cultured lineages can provide some temporal information on the P element population dynamics. In the present work we demonstrate that P element present the in wild-caught flies may be lost in the cultured fly lineages after some generations. This result is in accordance with the results of at least one published work and suggests that analysis of the cultured fly lineages may sometimes be unreliable in establishing historical trends in P element population dynamics, as the transposon may be occasionally lost, perhaps in the highly inbred lineages in which not all founding females carry it.

Introduction. *P* element is a DNA transposon 2.9 kb long present in the genomes of a number of drosophilid species and having been perhaps the most intensively studied transposable element over the last few decades [1]. Several reasons lie behind this remarkable popularity of *P* element. First of all, *P* elements have become a handy tool in *Drosophila* genetics [2], particularly as mutagenesis agents [3] and vector for the fly germline transformation [4]. Second, sometimes *P* element inflicts a drastic damage to the hosting genome as a result of its vehement transposition activity, and this behavior can be manipulated experimentally [5, 6]. This second feature makes *P* element a opportune model for studying transposition of cut-and-paste mobile elements. Besides, a number of *P* element horizontal transfer events have been recorded between different drosophilids, which has drawn the attention of evolutionary biologists. Once in the genome of a new species, the transposon quickly propagates over huge distances, invading many populations on rather large geographic scale [7–9]. Altogether these characteristics make *P* element a perfect model to study the evolution and function of DNA transposons.

A particularly fascinating thing about *P* element is that it seems to have invaded the genome of *Drosophila melanogaster* via horizontal transfer event less than a century ago and, since that time, has propagated all over the world in populations of the species [7, 10]. Multiple lines of evidence support this horizontal transfer scenario in principle. These include, among other, the identity of the *P* element DNA sequence in *D. melanogaster* and the putative evolutionary distant donor of the transposon, *D. willistoni*, combined with the absence of *P* element in species closely related to *D. melanogaster* [11, 12]. The timing of this transfer was initially deduced from an analysis of *D. melanogaster* laboratory lineages established from the wild flies caught in different times [7]. This analysis revealed that *P* cytotypes were absent from laboratory lineages established from the flies caught before 1950. *P* cytotype is a condition of cells that enables them to repress the activity of P element and results from a certain period of coevolution of *P* element and its hosting genome. Opposed to it is *M* cytotype, the primeval status of cells characteristic of flies that have not hosted *P* element in their genome or hosted it for a too short period. The presence of only *M* cytotypes in these lineages suggested that *P* element invaded



Electrophoregrams of *P* element PCR products: *a* – collected in 2008, second DNA extraction; *b* – collected in 2008, first DNA extraction (explained in the text); *c* – collected in 2009; *d* – collected in 2010. Natural fly populations: *1* – Uman, *2* – Pyriatyn, *3* – Varva, *4* – Lubny, *5* – Odesa, *8* – Magarach, *9* – Kyiv, *10* – Cooling Pond, *11* – Chornobyl; *6* – negative control (*Canton-S*), *7* – positive control (*Harwich*)

this fly species not long before 1950. It has been shown, however, that some aspects of laboratory culture, such as perhaps small population size, may act to eradicate *P* element from cultured lineages with time in a matter of several thousand generations [13].

This data did not undermine the cumulative evidence about the timing of *P* element invasion of *D. melanogaster* genome, but suggested that historical analysis of the lineages may hide some barely discernible pitfalls as a stand-alone technique for mobile element invasion analysis and should only be used in combination with other methods. To further investigate this problem and assess the number of culture generations that may matter in this type of analysis, we monitored the presence of *P* element in our laboratory lineages of *D. melanogaster* established from the flies collected from nature during three successive years. Our results suggest that *P* element may disappear from culture lineages on even shorter timescales within a matter of hundred generations.

Materials and Methods. Flies were collected during August 2008, 2009, and 2010 from 9 different locations (the cooling pond of the Chornobyl Nuclear Power Plant, the cities of Chornobyl, Kyiv, Varva, Lubny, Pyriatyn, Uman, Odessa, Yalta [Magarach]) distributed so that they represent a latitudinal cross-section of the territory of Ukraine (for the map refer to [14]). A total of 30 isofemale lines were established from each collected population (reviewed in [15]). In order to imitate the random nature of DNA sampling while analyzing old laboratory lineages, we prepared mixed DNA samples representing all isofemale lines available for each population.

Total DNA was extracted from adult individuals of each population using QIAamp DNA Micro Kit («Qiagen», USA).

We amplified a 437 bp region of *P* element DNA sequence by PCR using primers 5'-ACGTTTGCTTGTTGAGAGGA-3' and 5'-AACAGGACCTAACGCACAGT-3' specific to the region of *P* element ranging from the 41th to the 477th base. This region is believed to be part of all known types *P* element. 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 73 °C/10 min. Sequencing was performed in the Engencore sequencing lab of the University of South Carolina, USA, using the 3130 Genetic Analyzer («Applied Biosystems», USA).

For the sequence refer to [14] or NCBI (GenBank ID: HQ607781). All diagnostic PCRs were repeated three times and with proper positive and negative controls to ensure the absence of accidental mistakes.

As a *P* element-containing positive control, we used the laboratory drosophila strain *Harwich*. The wild-type laboratory strain *Canton-S* was used as a negative control (lacking *P* element).

Sequence alignment was performed using the Vector NTI software («Invitrogen», USA).

Results. All populations had been checked for the presence of *P* element within a week after collection and all were *P* element-positive [14]. We maintained fly isofemale lineages of 2008 collection from August, 2008 through August 2009, when the first DNA extractions were done. The

rest of 2008 collection was maintained up to November, 2010, when another round of extraction was performed. DNA from the lineages of 2009 and 2010 collection was extracted in November 2010. In order to imitate the random nature of DNA sampling while analyzing old laboratory lineages, we prepared mixed cultures of all isofemale lines available for each population and then extracted DNA from 10 randomly chosen flies of both sexes. Such a pooled random DNA sample was considered as being representative of the corresponding lineage or population and was, thus, notedly biased compared to any representative sample normally taken from a population or lineage. As a result, we had four DNA samples: two one-year old lineage DNA samples (collections: 2008 second extraction and 2009), one two-year old lineage DNA (collection: 2008 first extraction), and one three-month old lineage sample (collection: 2010). The results of diagnostic PCRs are shown in Figure.

As can be seen from Figure, most lineages contained *P* elements after three months to two years of laboratory cultivation. However, the lineages established from three populations (Pyriatyn, Magarach (Yalta), and Kyiv) collected in 2008 appeared to have lost *P* element after one year of laboratory maintenance (2008 first extraction samples).

Discussion. Our results are in accordance with that published by Engels [13] and, moreover, demonstrate that *P* element may be lost from the originally «*P* element-positive» populations within a much shorter period of laboratory cultivation than known before. The reasons that stay behind such a phenomenon may primarily lie in the fact that laboratory fly lineages become highly inbred with time, and chances are that the flies taken into tests may represent only one or a few of the wild caught flies which in fact did not contain *P* element. Such a scenario may well have been the case with our Ukrainian flies, as *P* element appeared in our populations relatively recently and may have not invaded all flies in some regions.

Nonetheless, our results in no way indicate that the inferences made by Kidwell [7] were wrong, as Kidwell dealt with cytotypes. Cytotype requires time to evolve after initial *P* element invasion, and this suggests that once no occurrences of *P* cytotype had been found in lineages established from flies collected before 1950, recent invasion was a plausible assumption. Our preliminary unpublished results, as well as those in [14], demonstrate that the populations which lineages lost *P* element in laboratory culture possessed *M*′ cytotype. This means that they have *P* element, but have not yet adapted to it. Such populations may, perhaps, be partially invaded by the transposon and some *P* element-free flies may have been involved in isofemale line establishment. In any case, however, our results suggest that historical analysis of old laboratory fly lineages can not be a stand-alone approach, as the low population numbers and inbreeding make lineages deviate from being representative of their natural ancestor populations and may produce unexpected stochastic effects in the behavior of *P* element in the fly genome.

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О ПЕРСИСТИРОВАНИИ *P* ЭЛЕМЕНТА В КУЛЬТИВИРУЕМЫХ ЛИНИЯХ *DROSOPHILA MELANOGASTER*

Известно, что *P* транспозон попал в геном *Drosophila melanogaster* в 50-х годах прошлого столетия результате горизонтального переноса от *D. willistoni*. Частично время инвазии было рассчитано на основании анализа культивируемых линий дрозофилы, которые происходят от диких особей, культивируемых как изосамковые линии задолго до анализа. Такой анализ показал, что *P* элемент отсутствовал в геномах культивируемых линий, которые основаны из особей, собранных в природе до 1950 г. Хотя гипотеза переноса *P* элемента подтверждена различными доказательствами и ее достоверность не вызывает сомнений, мы решили проверить, дает ли анализ культивируемых линий информацию о временных факторах популяционной динамики *P* элемента. В настоящей работе мы показываем, что *P* элемент, присутствующий в диких особях, может быть утрачен в культивируемых линиях через некоторое количество поколений. Такие

результаты согласуются с данными как минимум одной опубликованной работы и свидетельствуют о том, что анализ культивируемых линий дрозофилы не всегда надежен при воссоздании исторических трендов популяционной динамики *P* элемента, поскольку этот транспозон может быть случайным образом утерян, очевидно, вследствие высокой инбредности линий, у которых геномы не всех самок-основателей содержали его.

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ПРО ПЕРСИСТУВАННЯ *P* ЕЛЕМЕНТА В КУЛЬТИВОВАНИХ ЛІНІЯХ *DROSOPHILA MELANOGASTER*

Відомо, що *P* транспозон потрапив до геному *Drosophila melanogaster* у 50-х роках минулого століття в результаті горизонтального переносу від *D. willistoni*. Частково час інвазії був розрахований на основі аналізу культивованих ліній дрозофіли, що походять від диких особин, котрі культивувались у лабораторії як ізосамкові лінії задовго до аналізу. Такий аналіз показав, що *P* елемент був відсутній у геномах культивованих ліній, заснованих з особин, що зібрані у природі до 1950 р. Хоча гіпотеза переносу *P* елемента підтверджується різноманітними доказами та її достовірність не викликає сумніву, ми вирішили перевірити, чи дає аналіз культивованих ліній інформацію щодо часового фактора популяційної динаміки *P* елемента. В даній роботі ми показуємо, що *P* елемент, присутній у диких особин, може втрачатись в культивованих лініях через певну кількість поколінь. Такі результати узгоджуються з даними щонайменше однієї опублікованої роботи та свідчать про те, що аналіз культивованих ліній дрозофіли може інколи бути ненадійним для відтворення історичних трендів популяційної динаміки *P* елемента, оскільки цей транспозон може випадковим чином втрачатись, очевидно, через високу інбредність ліній, в яких геноми не всіх самиць-засновників містили його.

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