Comparison of methods for the determination of the transposition rate of mobile elements

Lyudmila P Zakharenko*
Institute of Cytology and Genetics; Novosibirsk, Russia

There is widespread interest in understanding the rate of transposable element movement within populations and between species. A recent study using interspecific crosses between D. buzzatii and D. koepferae indicated that transposition rates in hybrids may be quite high. However, we suggest caution should be taken in this interpretation since AFLP methods to detect transposition events may lead to overestimated rate estimates. Comparative analyses of genome instability received by different methods suggest that transposition rates can be higher in intraspecific crosses compared to interspecific crosses.

A genome-wide approach opens up the possibility of obtaining new information in the field of genome instability. Nevertheless, every method has its own restrictions. In our comments, we compare cytological, genetic and genome-wide molecular analyses of mobile element transposition rates in hybrid genomes.

The mobility of transposable elements (TEs) is one of the factors that induce spontaneous genetic instability. The transposition rate of TEs is difficult to study due to the rarity of events and polymorphic TEs locations, even in inbred strains. AFLP and FISH were used by Vela et al. to estimate the TEs transposition rate in interspecies hybrids between D. koepferae females and D. buzzatii male after subsequent 3 backcrosses between a fertile hybrid female and a D. buzzatii male. It was found a big difference in number of TEs insertions by using different techniques.

TEs Osvaldo, Helena and Galileo were detected by FISH as several hybridization sites on the salivary gland polytene chromosomes (12 hybridization site for Helena, no one for Osvaldo and one for Galileo in the case of D. koepferae; one hybridization site for Galileo, 5 for Helena and 4 for Osvaldo in the case of D. buzzatii), whereas the molecular method AFLP detected 2–5 dozen insertions per genome. The authors supposed that the difference in the TEs copy number studied by different methods appeared because FISH detects only euchromatic sites while AFLP can also detect heterochromatic sites. Nevertheless different TEs hybridize differently to chromocenter which mainly consists of heterochromatic blocks. Osvaldo hybridizes with chromocenter of both Drosophila species in comparison with Galileo and Helena. It means that we should find another explanation of discrepancy in data obtained by FISH and AFLP.

The difference in the TEs copy number found in hybrid genomes by FISH and AFLP approach can be explained by different sensitivities of these methods. The FISH analysis of the hobo, mdg1, DM412 and I-element transposition rates in the reference D. melanogaster genome can only realize usually sequences that have a base pair length of more than 1,000, although in this genome, there are several dozen smaller TE derivatives that were annotated.1,2 TE localized in chromocenter usually represented by short defective copies and can be recognized by FISH only if they are highly repeated. As usually hybridization of TE in chromocenter region produce diffuse signal instead of bands. This fact confirms in silico data that chromocenters accumulate short defective repetitive TEs variants.

Keywords: AFLP, FISH, genome instability, hybrid, transposition

Abbreviations: AFLP, amplified fragment length polymorphism; FISH, fluorescence in situ hybridization.

© Lyudmila P Zakharenko
*Correspondence to: Lyudmila P Zakharenko; Email: zakharlp@bionet.nsc.ru

Commentary on: Vela D, Fontdevila A, Vieira C, García Guerrero MP. A genome-wide survey of genetic instability by transposition in Drosophila hybrids. PLoS One 2014; 9:e88992; PMID: 24586475; http://dx.doi.org/10.1371/journal.pone.0088992
AFLP-based technique allows the simultaneous amplification of the TE insertions from a particular element which are identified by a ligation-mediated nested PCR that starts within the transposon and amplifies part of the flanking sequence. The polymorphic length of PCR products in AFLP analysis is a reflection of the polymorphism of adjacent TEs sequences. AFLP allows for the registration of short defective TEs variants, but most of short defective TEs variants cannot be recognized and moved by transposase, because most of them do not have both terminal repeats.

An example of the instability of gene singed in intraspecies hybrids is presented in Vela et al.’s article as a reflection of the rate of P-element transposition because of the excision of the P-element introduced in this gene. Meanwhile, it was found that mutations in the unstable sn were caused by the introduction of 2 P-elements oriented end-to-end or head-to-head. Another explanation of sn instability is as follows: “homologous recombination or slippage of a replication fork between these repeats, perhaps during attempted transposition, would also produce the observed structures.”

Changes in the number of bands in the AFLP analysis in interspecies hybrids can be caused not only by TE transpositions, but also by conversion, multiplication or recombination between defective TEs repeats or between their repeated neighbors. This is the case, then recombination between repeats adjacent to TE or recombination between different TEs can simulate TEs movement recognized by AFLP. By the way, Osvaldo is the most movable TE according to AFLP technique and the only TE that strongly hybridizes with chromocenter.

It was notice also by Vela et al. a difference in the number of AFLP markers between the different flies of parental lines in spite of the fact that both stocks correspond to inbred lines maintained by brother–sister mating for several years. The number of total Galileo insertions in 2 different families was 41, 43 and 45 in the case of D. buzzatii and D. koepferae respectively (table 5). According to Figure 1 from Vela et al. article paternal insertion sites are nearly summarized in hybrids because AFLP markers are not coincide in analyzed species. However family 1 in backcross 1 has only 25 total Galileo insertions. The biggest number (52) of total Galileo insertions was found in family 40. It is not clear, what is the impact of polymorphic pattern of parental TEs in AFLP result if backcross1 was carried out by mass crossing.

A similar divergence between the genome-wide and cytological data in the analysis of the rate of TE transposition was found in Drosophila melanogaster.
intraspecies crosses. Authors directly mapped new transposon insertions by paired-end deep sequencing of ovarian DNA. Genetic studies detected approximately one new P-element insertion/generation. By contrast, genome sequence analysis of dysgenic ovaries devoted viable eggs, revealed approximately 15 new insertions in a single generation. The length of P-elements was not determined as in the Vela et al.’s article. The rate of P-element transposition was about $10^{-2}$ and $10^{-1}$ according to hybridization in situ and genome-wide analysis respectively. Some other TEs also were very movable in intraspecies crosses. The frequency of TEs transpositions in interspecies hybrids varies from $10^{-2}$ to $10^{-4}$ per genome per generation according to AFLP-based technique.

**Conclusion**

Genome-wide methods identify increases in genome instability in inter- and intra-species hybrids, but overestimate TEs transposition rates by at least one order of magnitude in comparison with FISH. This is because genome-wide methods can recognize not only movable TE, but also short defective derivatives, which have lost the potential to change positions. Instability which is recognized by AFLP technique can reflect changes of TEs position that happens mainly without transposase participation. Recombination between repeats may play a significant role in appearance of hybrid genome instability. The genome instability in intraspecies crosses is at least one order of magnitude higher than in interspecies hybrids if take into account genome-wide approaches.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

In silico analysis was done by M. Perepelkina.

**Funding**

This work is supported partially by grant RFBR 14-04-00929 and Base Project VI.53.1.2.

**References**

1. Zakharenko LP, Kovalenko LV, Mai S. Fluorescence in situ hybridization analysis of hobo, mdg1 and Dm412 transposable elements reveals genomic instability following the Drosophila melanogaster genome sequencing. Heredity (Edinb) 2007; 99:525-30; PMID:17622267; http://dx.doi.org/10.1038/hdy.6801029

2. Moschetti R, Dimitri P, Caiazi R, Junakovic N. Genomic instability of I elements in Drosophila melanogaster in absence of dysgenic crosses. PLoS One 2010; 5(10). pii: e13142; PMID:20957225; http://dx.doi.org/10.1371/journal.pone.0013142

3. Rasmussen KE, Raymond JD, Simmons MJ. Repression of hybrid dysgenesis in Drosophila melanogaster by individual naturally occurring P elements. Genetics 1993; 133:605-22; PMID:8384145

4. Reisha H, Rubin GM, O’Hare K. P element insertions and rearrangements at the singed locus of Drosophila melanogaster. Genetics 1988, 119:75-83; PMID:2840331

5. Norris ES, Woodruff RC. Visible mutations induced by P-M hybrid dysgenesis in Drosophila melanogaster result predominantly from P element insertions. Mutat Res 1992; 260:63-72; PMID:1381472; http://dx.doi.org/10.1016/0027-5107(92)90161-T

6. Eggleston WB, Rim NR, Lim JK. Molecular characterization of hobo-mediated inversions in Drosophila melanogaster. Genetics 1996, 144:647-56; PMID:8889527

7. Zakharenko L, Zakharov I, Romanova O, Voloshina M, Gracheva E, Kochieva EZ, Golubovskil MD, Georgiev PG. “Mode for mutation” in the natural population of Drosophila melanogaster from Uman is caused by distribution of a hobo-induced inversion in the regulatory region of the yellow gene. Genetika (Russian) 2000; 36:740-8; PMID:10923255.

8. Stevison LS, Hocht KB, Noor MA. Effects of inversions on within- and between-species recombination and divergence. Genome Biol Evol 2011; 3:830-41; PMID:21828374; http://dx.doi.org/10.1093/gbe/evr081

9. Khurana JS, Wang J, Xu J, Koppersch BS, Thomson TC, Nowosielska A, Li C, Zamore PD, Weng Z, et al. Adaptation to P element transposon invasion in Drosophila melanogaster. Cell 2011; 147:1551-63; PMID:21967376; http://dx.doi.org/10.1016/j.cell.2011.11.042