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

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There is wide-spread interest in understanding the rate of transposable element movement within populations and between species. A recent study using interprecific 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.

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

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

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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.

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 variant, 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[3] 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.[4,5] 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".[4] Thus, *singed* instability may not be a reliable indicator of *P*-element transposition. It was shown also that the instability in *Notch*[6] and *yellow*-2[7] was caused by recombination between 2 TEs. Hypermutability in *yellow2* coincided with the multiplication of inversion appeared in the regulatory region between 2 *hobo* transposable elements and recombination between repeats.[7]

Recombination frequency increases also in heterozygote in pericentromeric regions[8] where defective TEs and other repeats are concentrated. As was mentioned by authors of commented article "new AFLP hybrid markers detected in *Amaranthus* hybrids have homology with TEs associated to the mobilization of repetitive DNA." We found an example of distribution of 3 kb degenerative repeat between TEs in annotated *y; cn bw sp D. melanogaster* genome (**Fig. 1**). The members of this degenerative family are divided into 2 groups according to there's similarity and are distributed between different couples of short defective TEs (*jockey* and *Rt1*; *Stalker* and *invader*). Members of one group are situated mainly between *jockey* and *Rt1* couples, but members of other group are localized mainly between *Stalker* and *invader* couples. Appearance of such structure and multiplication of TEs could be result of recombination between these 3 kb repeats.

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. If 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 42, 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*

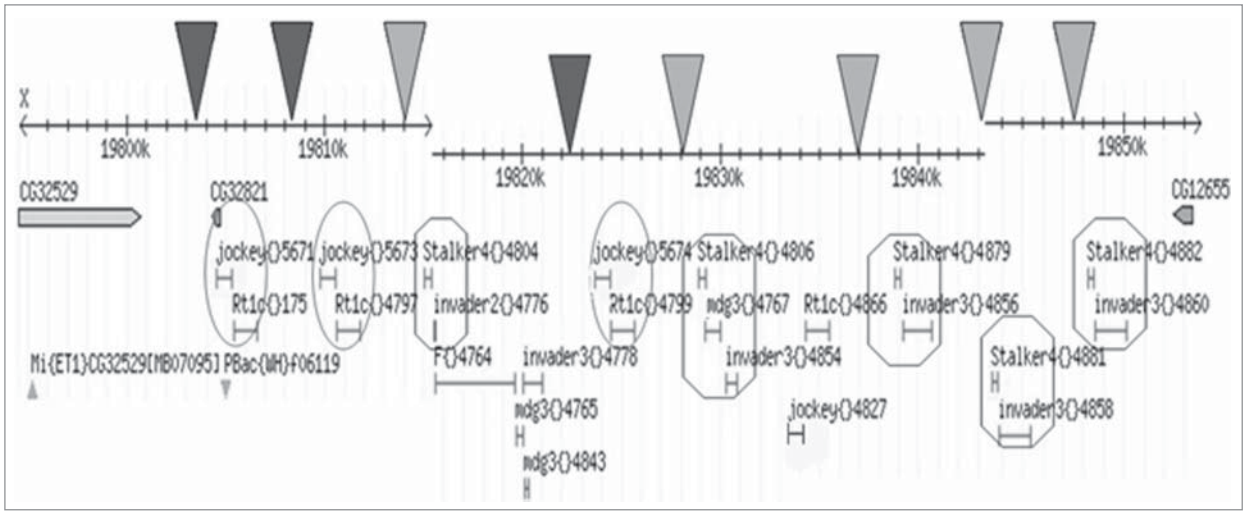

Figure 1. Distribution of 3 kb repeats between couples of TEs (*jockey* and *Rt1* in oval; *Stalker* and *invader* in poligon) on the X-chromosome of *y; cn bw sp D. melanogaster* genome (*in silico* data was obtained from Flybase). Arrow heads show the distribution of 3 kb repeats. Color of arrow heads reflects the groups of similar repeats.

intraspecies crosses.[9] Authors directly mapped new transposon insertions by paired-end deep sequencing of ovarian DNA. Genetic studies detected approximately one new *P-element* insertion/genome/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.

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