

# Interspecific hybridization as a genomic stressor inducing mobilization of transposable elements in *Drosophila*

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**Keywords:** *Drosophila*, transposable elements, interspecific hybridization, transposition, genomic instability, epigenetic factors

**Abbreviations:** TEs, transposable elements; AFLP, amplified fragments length polymorphism; piRNAs, Piwi-interacting RNAs; siRNAs, small interfering RNAs

Submitted: 07/15/2014

Revised: 08/07/2014

Accepted: 08/08/2014

Published Online: 08/08/2014

Citation: Pilar García Guerreiro M. Interspecific hybridization as a genomic stressor inducing mobilization of transposable elements in *Drosophila*. Mobile Genetic Elements 2014; 4:e34394; <http://dx.doi.org/10.4161/mge.34394>

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Commentary to: Vela D, Fontdevila A, Vieira C, García Guerreiro 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>

**T**ransposable elements (TEs) are DNA sequences able to be mobilized in host genomes. They are currently recognized as the major mutation inducers because of their insertion in the target, their effect on neighboring regions, or their ectopic recombination. A large number of factors including chemical and physical factors as well as intraspecific crosses have traditionally been identified as inducers of transposition. Besides environmental factors, interspecific crosses have also been proposed as promoters of transposition of particular TEs in plants and different animals. Our previous published work includes a genome-wide survey with the set of genomic TEs and shows that interspecific hybridization between the species *Drosophila buzzatii* and *Drosophila koepferae* induces genomic instability by transposition bursts. A high percentage of this instability corresponds to TEs belonging to classes I and II. The detailed study of three TEs (*Oswaldo*, *Helena*, and *Galileo*), representative of the different TE families, shows an increase of transposition in hybrids compared with parental species, that varies depending on the element. This study suggests ample variation in TE regulation mechanisms and the question is why this variation occurs. Interspecific hybridization is a genomic stressor that disrupts the stability of TEs probably contributing to a relaxation of the mechanisms controlling TEs in the *Drosophila* genome. In this commentary paper we will discuss these results and the molecular mechanisms that could explain these increases of transposition rates observed in interspecific *Drosophila* hybrids.

## *D. buzzatii*, *D. koepferae*, and their Hybrids

*Drosophila buzzatii* and *Drosophila koepferae* are two cactophilic sibling species, belonging to the repleta group,<sup>1</sup> morphologically undistinguishable except by the male genitalia. They have an overlapping distribution in the arid zones of southern South America, although *D. buzzatii* has a wider geographical distribution compared with *D. koepferae*, which is mainly restricted to the oriental side of the Andes Mountains.<sup>2</sup> Moreover, they present a partial ecological isolation because *D. buzzatii* breeds and feeds on cacti from genus *Opuntia* whereas *D. koepferae* prefers columnar cacti from *Cereus* and *Trichocereus* genus.<sup>2</sup> Although recent studies have provided evidences of recent gene flow between both species,<sup>3</sup> we know they present reproductive isolation. Hybrids have not been found in the wild yet, due probably to the absence of a molecular tool allowing their quick identification. In laboratory, interspecific crosses are possible as *D. buzzatii* males can mate *D. koepferae* females and female hybrid offspring can be backcrossed to *D. buzzatii* males.<sup>4</sup> This potential of natural hybridization makes these species an excellent model for speciation studies by transposition, particularly because very early studies had already showed increases of transposition rates of *Oswaldo*, the best characterized retrotransposon in *D. buzzatii*,<sup>5</sup> in hybrids compared with parental species.<sup>6</sup>

## TE mobilization in *Drosophila* Interspecific Hybrids

The most numerous and best documented cases of TE mobilization after interspecific hybridization were reported in plants,<sup>7-9</sup> where hybrids are easier to obtain and study than in animals in which the number of offspring is low making the crosses very difficult. However, now some examples begin to be available in animals: one of the best known cases is the kangaroo where increases of transposition and centromere expansion were observed in hybrids.<sup>10,11</sup> The first direct observation of increases of transposition in hybrids between *D. buzzatii* and *D. koepferae* was reported by Labrador and Fontdevila<sup>12</sup> with the retrotransposon *Oswaldo*. These results were confirmed by a second experiment<sup>6</sup> that included a larger sample size and a robust quantitative test. The next published work<sup>13</sup> in *Drosophila* referred to hybrids between *D. melanogaster* and *D. simulans* and showed a widespread depression of different TE families in F1 hybrids. Our recent published work,<sup>14</sup> on *D. buzzatii* and *D. koepferae*, analyzes by the first time the set of genomic elements by a genome-wide approach. The hybrids were followed by four successive generations (F1 hybrids and three backcrosses) and stocks used did not contain mutations that rescued sterility in F1, reproducing the natural conditions of both species. We observed that a total of 33 TEs belonging to 14 TE families of class I and II were mobilized in hybrid genomes compared with parental species, where only one TE seemed to be mobilized. Likely the number of mobilized elements is greater than observed, due to the low size of AFLP bands and the lack of a reference sequenced genome, some elements could have been unnoticed. However, the number of elements in this study constitutes a representative part of the parental species genome. The detailed study of *Oswaldo*, *Helena*, and *Galileo*, representative of LTR-retrotransposons, non-LTR-retrotransposons and transposons respectively, showed an increase of transposition rates in hybrids compared with parental species, reaching in some cases one order of magnitude greater.

It is noteworthy that in these experiments differences were observed in the number of TEs mobilized as well as in the transposition rates across the different generations and hybrid families (hybrid crosses replicas). These results point first to the different mechanisms of regulation between elements and, second, that the portion of genome introgressed in hybrids could play an important role in TE activation. It is important to note that the percentage of *D. buzzatii* genome introgressed in hybrids is increasing in each backcross with *D. buzzatii* parental males, and the region of genome is different between families. Differences in hybrid instability or gene misexpression between generations of hybrids are not rare and have already been reported in plants<sup>15</sup> and mouse.<sup>16</sup>

Another important point is the trend to a higher increase of transposition rates in males than in females, even if the differences were only significant in one family. We ignore how the elements are awakened in hybrids or why that is especially relevant in males, as seen mainly for *Oswaldo* retrotransposon.<sup>14</sup> We think that most transposition events occurring in hybrids happened in female germline because a higher number of insertions are segregating along hybrid generations and it is hybrid females which are repeatedly crossed with *D. buzzatii* males. Transpositions observed in hybrid males could correspond to events in somatic cells or/and in hybrid male germinal tissues that do not contribute to the next generation because hybrid males are sterile. Out of the hybrid context, cases of *copia*<sup>17,18</sup> transpositions or expression of *412*,<sup>19</sup> *micropia*,<sup>20</sup> and *1731*<sup>21</sup> have been reported in *Drosophila* males suggesting regulation mechanisms different from females.

### Why TE Activity Increases in *Drosophila* Hybrids?

The mechanism that induces transposition in hybrids is largely unknown and to date only an experimental study has addressed this issue in *Drosophila*.<sup>13</sup> But, the similarities between events (sterility and transposition) occurring during interspecific hybridization and hybrid

dysgenesis<sup>22</sup> suggest a possible parallelism. During the *Drosophila* hybrid dysgenesis, by crosses between wild and laboratory strains, sterility and increases of transposition were observed in the F1 offspring as a consequence of the activation of male-derived TEs. This happened because maternally contributed Piwi-interacting RNAs (piRNA) were unable to repress TEs paternally derived. In the same way, during interspecific hybridization between *Arabidopsis* species, the parental copies of *Athila* TE, normally silenced in heterochromatin, were expressed in hybrids.<sup>23</sup> The authors hypothesize that the activation of the element could be due to the insufficient production of small interference RNAs (siRNAs) in females or their low specificity because of the sequence divergence between the two *Arabidopsis* species used in crosses. It is proposed that hybrid genetic dysfunctions occurred not only by the sequence divergence of genes in the two species but also by a large variety of mechanisms related with integrity and maintenance of chromatin.<sup>24</sup> It is well known that the epigenetic control can affect gene expression and TE mobilization, a phenomenon extensively studied in *Drosophila* especially in the case of genes of the *piwi* pathway responsible for defending germline from TE proliferation.<sup>25</sup> Kelleher et al.<sup>13</sup> found both TE misregulation and aberrant piRNA production in interspecific hybrids between *D. melanogaster* and *D. simulans*, suggesting an adaptive divergence of piRNA pathway genes rather than differences in piRNAs derived from TEs in the species under study.

Increases of transposition were observed in our *D. buzzatii/D. koepferae* hybrids and raises the question about the involvement of TE mobilization in hybrid sterility and the molecular causes. Previous genetic work, with these same hybrids, proposes that male hybrid sterility results from the cumulative action of many interacting genes of minor effects.<sup>26</sup> We ignore the direct impact of TE derepression in fecundity of *D. buzzatii/D. koepferae* hybrids but the divergence between alleles of piRNA pathway could contribute both to sterility and TE mobilization. In *Drosophila*, proteins encoded by these genes participate in piRNA biogenesis, transposon silencing,<sup>27</sup>

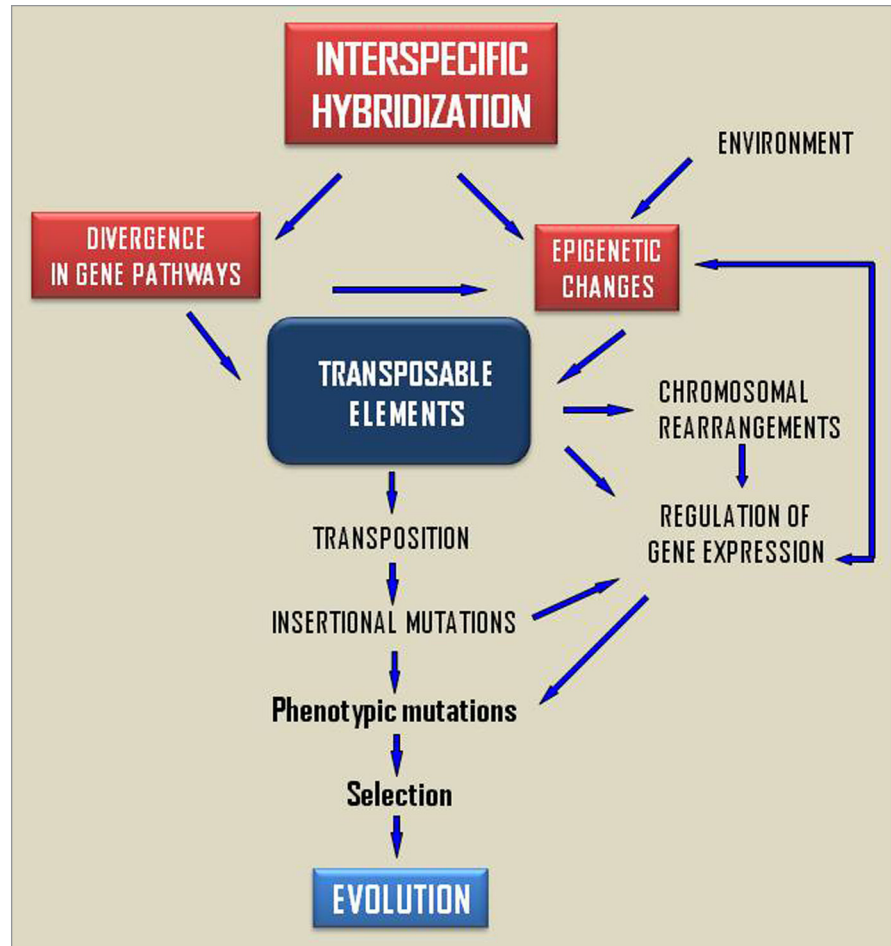
and germline stem cell (GSC) self-renewal in both males and females<sup>28</sup> and are important in the maintaining of fertility.<sup>29,30</sup> Mutations in Aubergine gene (a member of piRNA pathway) lead to accumulation of retrotransposons on ovaries and testes, and *Stellate* transcripts in testes that are associated to male sterility.<sup>29</sup> In view of the importance of these mentioned genes I suggest that the hybrid sterility is not directly caused by transposition but that could be a consequence of adaptive divergence of *piwi* pathway genes. However, we cannot completely discard the possibility that TE mobilization in hybrids affect sterility because of their capability of neighboring genes misregulation.<sup>31</sup>

On the other hand to explain TE mobilization, it is known that piRNAs silencing transposons come from heterochromatic TE-rich regions named piRNA clusters.<sup>32,33</sup> For many TEs the maternal deposition of TE specific piRNAs is critical for their propagation and, in the case of interspecific hybrid, if they do not inherit the piRNA clusters, or the elements inserted inside have diverged between the two species, the amount of piRNAs would not be sufficient to repress TEs. This point could be the cause of differences in the amount of TE mobilized through different hybridization generations and/or families observed in our work.

To summarize, the **Figure 1** depicts putative scenarios leading to transposition in hybrids and their implications in speciation processes. The genomic “shock” by interspecific hybridization leads to an epigenetic reprogramming<sup>24</sup> and a TE release associated to mutations and new gene regulatory ways. Some of these mutations could be fixed by selection in a novel environment contributing to speciation processes. There is increasing evidences suggesting that TEs had and have a promising role in evolutionary processes. Transposition bursts ensuing hybridization have been suggested as makers of rapid genome reorganizations and source of evolutionary innovations,<sup>34</sup> notwithstanding

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**Figure 1.** Ways of TE activation in hybrids and the putative role in speciation events.

more empirical work will be necessary to address these questions conclusively. The recent advances in the knowledge of TE silencing associated to interference RNAs (RNAi and piRNA) could open new ways to the understanding of TE activation in hybrids. Provided that the genomic context could affect epigenetic regulation, a way to disentangle the mechanisms implicated in TEs deregulation in hybrids could consist of comparing the small RNA content of hybrids and parental species. This study could be completed with the analysis of the expression of piRNA pathway genes in order to detected putative expression patterns altered in hybrids compared with parental species.

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## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

I want to thank O Cuba for reviewing the English and A Fontdevila, D Vela, and C Vieira as authors of the Plos One previous published work that thanks to their results, this Commentary has been possible. This work was supported by research grants CGL2009-12912-C03-01 and CGL2010-15395 from the Ministerio de Ciencia e Innovación (Spain), and grants 2009SGR 636 from Generalitat de Catalunya to the Grup de Biologia Evolutiva.

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