Distribution, evolution, and diversity of retrotransposons at the flamenco locus reflect the regulatory properties of piRNA clusters

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Most of our understanding of Drosophila heterochromatin structure and evolution has come from the annotation of heterochromatin from the isogenic y; cn bw sp strain. However, almost nothing is known about the heterochromatin’s structural dynamics and evolution. Here, we focus on a 180-kb heterochromatic locus producing Piwi-interacting RNAs (piRNAs) called flamenco (flam), known to be responsible for the control of at least three transposable elements (TEs). We report its detailed structure in three different Drosophila lines chosen according to their capacity to repress or not to repress the expression of two retrotransposons named ZAM and Idefix, and we show that they display high structural diversity. Numerous rearrangements due to homologous and nonhomologous recombination, deletions and segmental duplications, and loss and gain of TEs are diverse sources of active genomic variation at this locus. Notably, we evidence a correlation between the presence of ZAM and Idefix in this piRNA cluster and their silencing. They are absent from flam in the strain where they are derepressed. We show that, unexpectedly, more than half of the flam locus results from recent TE insertions and that most of the elements concerned are prone to horizontal transfer between species of the melanogaster subgroup. We build a model showing how such high and constant dynamics of a piRNA master locus open the way to continual emergence of new patterns of piRNA biogenesis leading to changes in the level of transposition control.

RNAi | gene silencing | epigenetics

Over the course of evolution, transposable elements (TEs) have accumulated in the genomes of eukaryotes, where they can account for up to 85% of the DNA (1). Most of these sequences have lost their ability to transpose. They are now stable components of the genomes. Their conservation throughout evolution suggests that they may confer advantageous effects to their hosts. However, transposition of the copies that remain functional could generate deleterious mutations if they were not severely repressed by their host. RNAi, which is a gene-silencing mechanism triggered by small RNAs (reviewed in ref. 2), has been identified as being the main cellular machinery involved in the “taming” of TEs (reviewed in refs. 3–5). RNAi pathways involve small RNAs of diverse families. Among them, Piwi-interacting RNAs (piRNAs) have been shown to be involved in TE silencing in the Drosophila ovary. These piRNAs, 23–29 nt long, are bound by the Argonaute proteins Piwi, Argonaute 3, or Aubergine. They are produced by discrete genomic loci named piRNA clusters, which have been described as containing vestiges of TEs (6). One of these loci, the flamenco (flam) locus, extends over 180 kilobases (kb) on the Drosophila X chromosome. It is proximal to the DISCO interacting protein 1 gene (DIP1) and close to pericentric heterochromatin. Before the identification of piRNAs, this locus had been shown to regulate the Gypsy retrotransposon (7, 8). Desset et al. (9) identified a locus called COM responsible for the control of ZAM and Idefix retrotransposons. Later, flam and COM were shown to be one and the same large master locus for regulation of at least these three retrotransposons: ZAM, Idefix, and Gypsy (reviewed in ref. 10). Control of different retrotransposons by the flam locus had been shown for diverse flam alleles: for ZAM and Idefix in the Rev line (9) and for Gypsy and ZAM in flam mutant lines (11). Brennecke et al. (6) showed the potential for the flam cluster to produce 79% of all ovarian piRNAs that target ZAM and 30% and 33% of those matching Idefix and Gypsy, respectively. It was further reported that a functional pathway linking flam piRNA to Gypsy suppression. Indeed, a substantial reduction of piRNA homologous to Gypsy was observed in flam mutant lines that are permissive to Gypsy transposition (12). Still, the molecular rules allowing this locus to control several retrotransposons are far from being understood.

In previous work, we isolated Drosophila lines in which ZAM and Idefix are either silenced (i.e., “stable”) or derepressed (i.e., “unstable”). Over the course of a P-element mediated mutagenesis

Significance

Control of transposable elements (TEs) by RNAi has a large impact on genome evolution in higher eucaryotes. In this paper, we study in detail a Piwi-interacting RNA (piRNA)-producing locus of Drosophila melanogaster, flamenco (flam), known to be responsible for the control of at least three retrotransposons by RNAi. We demonstrate the high structural dynamics of the flam locus resulting in loss and gain of TEs and establish a link between such structural variations and its ability to silence retrotransposons. We show that flam is a trap for TEs coming in by horizontal transfer from other Drosophila species. Overall, our data give unique insights into piRNA cluster regulatory properties, their role in evolution, and expansion and taming of TEs.

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performed on a stable line called w^{1B6}, we isolated a line, Rev, derived from w^{1B6}, in which ZAM and Idefix are both derepressed (13–15). Novel mobilizations are regularly observed, and reporter genes containing ZAM or Idefix fragments are expressed in the Rev line, although silenced in w^{1B6}. We further reported that the observed release in ZAM and Idefix silencing was due to an unidentified mutation having affected the 

(ZAM) indicates that the solo-LTR does not locus. This might indicate that the solo-LTR does not locus. 

Bergman et al. (16) laid out the hypothesis that [p-heterochromatic TE nests will act as transposon traps for new TE invasions by horizontal transfer providing an “adaptive immunity” to the host genome. Here, we wondered whether specifically piRNA loci like 

flam might act as a trap for TEs. If so, any trapped TE that inserted into a piRNA cluster should immediately be silenced and further transposition events should not occur. Then, a piRNA locus would be predicted to contain only one copy of a TE per family. To test this model, we first revisited the annotation of the 

flam locus. We saw that, as predicted, most of the TEs in the 

flam locus are present only once. More than half of the 

flam locus is made up of recent TEs, and two-thirds of them arose from horizontal transfer, confirming the trap model. We then evaluate the structure of the 

flam region in permissive and repressive strains. Our data indicate that strains permissive for the mobility of 

ZAM and Idefix display large deletions that removed ZAM and Idefix sequences present in 

flam. These results reveal a tight correlation between the presence of these retrotransposons in this locus and their silencing. They also highlight how transposition bursts may occur due to the high molecular dynamics of piRNA clusters like 

flam.

Results and Discussion

Most of the TEs in 

flam Are Present Only Once in This Locus. We predicted that if a TE is silenced as soon as it inserts into the 

flam locus, it should be present only once in 

flam because further transposition events should simply not occur. However, the current annotation [ref. 17 and Flybase (http://flybase.org)] appeared to contradict this prediction. Actually, there were several copies annotated for the same TEs (i.e., for 

ZAM, Idefix, Stalker, Stalker2, Stalker4, gypsy6, springer, mdg1, F-element). Several hypotheses could be made: Either multiple insertions of these TEs occurred, contradicting our prediction, or single insertions occurred, followed by duplication, or single insertions were misrepresented as multiple insertions because of erroneous annotation.

We conducted a bioinformatics analysis of the 

flam locus to check these hypotheses. To generate reannotation the 180-kb sequence of the 

flam locus of the sequenced Iso1A strain was analyzed by CENSOR using Repbase (www.girinst.org) (18, 19). The whole sequence was also aligned to itself, in both orientations, to analyze sequence redundancy. Among the 52 different TEs that are present at this locus and present in Repbase, we found 49 being present as a unique copy in 

flam, supporting the single-insertion prediction. Only the F-element, a long interspersed nucleotidic element (LINE), and the LTR retrotransposons mdg1 and Stalker4 are multicopy. Two F-elements are present in 

flam, one full-length copy inserted in the Plus orientation (distal to proximal) and a second truncated copy inserted in the Minus (proximal to distal) orientation (Fig. 1 and Table S1). Six distinct mdg1 copies inserted in both orientations have 97–99.4% sequence identity with the referenced element. These 

mdg1 copies are mainly clustered in one site, as is observed in another locus from chromosome 2 (2R:6509912..6524619, Drosophila melanogaster genome release 5.1).

We identified segmental duplication events and found that several mdg1 and Stalker4 fragments show the same breakpoints as other mdg1 and Stalker4 fragments in the locus [Table S1; mdg1(flam)4, 5, and 6; Stalker4(flam)2, 3, 7, and 8]. Segmental duplications in the 

flam region had also been shown by Bergman et al. (16), who proposed that they play an important role in the genesis of TE-rich regions of the genome.

Another case of sequence redundancy concerns solo-LTRs. Actually, we observed TEs for which, in addition to one full-length copy, a solo-LTR is present elsewhere in the 

flam locus. This is the case for mdg1 and an element that we later identified as being Pifo (see below). The presence of the Pifo solo-LTR in addition to a full-length Pifo indicates that Pifo inserted twice in the 

flam locus. This might indicate that the solo-LTR does not generate enough piRNA to repress Pifo transposition.

The presence of several copies in 

flam suggests that the F-element, mdg1, and Stalker4 might not be silenced by their presence in 

flam. Actually, even if some of the mdg1 and Stalker4 copies appear to originate from segmental duplication, others seem to arise from reiterated insertions. For the remaining 49 TEs present in 

flam, our data confirm the single-insertion prediction supporting that these TEs are candidates to be silenced by their presence in this particular piRNA cluster.

In Silico Analysis of the 

flam Locus Reveals a Large Number of New TEs and Gives a Basis for New Annotation of the Locus. Although the vast majority of the 

flam TEs are single copy in this locus, as shown by our reannotation above, some of them had been previously annotated as if they were present several times in 

flam. This was also the case for 

ZAM and Idefix (flam annotation is provided in Flybase and in ref. 17). Because we aimed to analyze 

ZAM- and Idefix-related elements in 

flam in silenced and derepressed Drosophila strains, we first had to elucidate the quality of the existing 

flam annotation. Moreover, annotations offered a glimpse of the 

flam locus as a genomic region highly fragmented and mainly composed of degenerated vestiges of TEs. To verify that this was indeed the case, we first used CENSOR, utilizing Repbase to extract all 

flam sequences that display less than 95% sequence identity with known TEs from D. melanogaster, corresponding to

![Fig. 1. Structure of the 

flam piRNA cluster in D. melanogaster. Recent TEs are presented in the upper part, and ancient elements are shown as the base line of the locus. Only recent elements, ZAM, Idefix, and the repeated Stalker4 copies, are shown individually (a detailed analysis of all TEs in 

flam is presented in Table S1). The centromere of the X chromosome is on the right-hand (proximal) side. The sense of transcription for the DIP1 gene and orientation of the TEs are indicated by arrows.](image)
Fig. 2. Proposed structure of *flam* and *cluster_17* in Iso1A, *w*¹⁶⁶, and Rev strains. Light gray boxes represent sections of *flam* (Left) and *cluster_17* (Right) that are present in Iso1A, *w*¹⁶⁶, or Rev. ZAM and Idefix sequences are shown in black, deleted sections are shown as missing zones (white), zones that might be present are shown as short vertical bars, and the segmental duplication of *flam* in *cluster_17* is indicated.

more than one-third of the *flam* locus. With these sequences, we searched for homologous sequences in the genomes of other *Drosophila* species (BLAST at http://flybase.org).

By this approach, we identified nine retrotransposons so far undescribed and six for which similar but not identical elements had been described (*Gypsy2S*, *Pifo*, *GYPSY6S*, *mdg1_DSe*, *Gypsy20S_DYa*, and *G6-fl*). These TEs are reported in Table S2 (their sequences are provided in Dataset S1). All have significant coding capacities. They can be found within the melanogaster subgroup in species such as *D. melanogaster*, *Drosophila simulans*, *Drosophila sechellia*, and *Drosophila erecta*. However, species outside of this subgroup, such as *D. melanogaster*, *Drosophila simulans*, *Drosophila pseudoobscura*, and *Drosophila persimilis*, are devoid of these TEs. The general map of the *flam* locus is presented in Fig. 1, and three interesting results may be pointed out. First, several regions that were annotated as being fragments of different TEs, or not annotated at all, correspond to one and the same TE in our revised annotation (Tables S1 and S2). As an example, a region that presented some homology with different fragments of *Gypsy10* (92% sequence identity) was revealed to display 99.2% sequence identity over the entire length with an element that we identified in *D. sechellia* (Table S2). We named this element *Philiippo*. Second, a region that was not annotated in *Flybase* showed more than 99% sequence identity with *Gypsy2* but seemed to be interrupted by an *HMSBEAGLE* fragment (Table S1). This element was revealed to be a full-length, entirely coding *Gypsy2* element that we named *Gypsy2S* (Table S2). Third, sequences that are not annotated in *Flybase* but had some limited sequence identity with *ZAM* and *Tirant* fragments correspond in fact to a newly identified element that we named *Agorino* and a *Pifo* element. *Pifo* was previously identified in *Drosophila yokuba* (20) and a 4,414-bp degenerate *Pifo* in *D. melanogaster* (21). Consequently, only one vestige of *ZAM* is present at position X:21649838..21655021. The same is true for *Idefix*; a single copy is found in *flam* at position X:21663930..21672608 (Fig. 1 and Table S1).

These results show that most of the sequences composing *flam* are long TE copies and confirm that some elements annotated as being present several times are present only once using our reannotation based on improved query sequences. Moreover, interspecies in silico analysis using *flam* sequence as a starting point allowed the identification of previously undescribed TEs within the melanogaster subgroup.

Structural Modifications Affecting *flam* May Explain Differential Silencing of TEs in Permissive and Nonpermissive Strains. We aimed to determine what makes the difference between *Drosophila* strains that are permissive or nonpermissive to the expression of particular retrotransposons, *ZAM* and *Idefix* in this case. It was already known that *ZAM* and *Idefix* expression depends on the *flam* locus, but nothing was established on how this works at the molecular level.

Three lines were analyzed: one permissive strain, *Rev*, and two nonpermissive strains, *w*¹⁶⁶ and the sequenced isogenic *cn bw sp* strain (here referenced as the Iso1A strain).

The genomic structure of *flam* was first examined through PCR amplifications. Primer design was inspired by the work of de La Roche Saint André and Bregliano (22), which showed how to amplify specifically one of many repeated sequences based on one nucleotide difference. Although most of the region of *flam* corresponds to repeated sequences of the *Drosophila* genome, we were able to build a collection of more than 180 primers distributed along the 180-kb locus (primer sequences are provided in Dataset S2). Twenty-five PCR DNA fragments all along the locus were analyzed in the first set of amplifications. When differences were observed, the regions concerned were analyzed in detail by other PCR amplifications and sequenced.

The first difference between the Iso1A strain and both the *w*¹⁶⁶ and Rev lines was revealed in region X:21511046..21526566. This region encompasses a 412 bp element inserted within a *Stalker2* element (Fig. 1). These two elements are absent both from *w*¹⁶⁶ and Rev (Fig. 2). PCR and sequencing of this region revealed that the target site of *Stalker2* and 412 insertion in Iso1A is clearly an empty site in *w*¹⁶⁶ and Rev [i.e., without any relics of 412 or *Stalker2* sequences (compare with GenBank accession nos. KF364662 and KF364663, respectively)]. This result indicates that *Stalker2* and 412 recently jumped into this position in the Iso1A strain.

Second, two short deletions found around the same region, one in *w*¹⁶⁶ and one in Rev: positions X:21527735..21527905 were found deleted in *w*¹⁶⁶, and positions X:21526698..21526797 are absent in Rev (compare with GenBank accession nos. KF364664 and KF364663, respectively). The fact that each of these deletions exists in one strain but not in the other indicates that they occurred after the isolation of Rev from *w*¹⁶⁶ some 20 years ago.

Most importantly, the third difference observed in the *flam* structure between the Iso1A, *w*¹⁶⁶, and Rev lines pointed out the potential relationship between its structure and its TE-silencing properties. This difference was found within the region...
X:21638001..21684449 containing the only ZAM and Idefix copies present in flam as reported above. Both are Minus-orientated. The ZAM copy shows significant homology to the active copy, with 94.1% sequence identity extending over 4,786 bp with an internal deletion from positions 4,298 and 6,968 (Repbase ZAM I sequence). Its sequence is interrupted by a Gypsy4-like solo-LTR. The Idefix copy has 92% sequence identity with canonical Idefix. This copy is fragmented by deletion of Idefix sequences and foreign insertions (Table S3). All these Idefix fragments add up to 3,150 bp distributed over 8,679 bp. PCR amplifications in the region containing ZAM and Idefix copies were successful in Iso1A and w110r6 but did not give any amplified fragments in Rev (Fig. S1). By inverse PCR, we amplified the DNA fragment adjacent to the supposed breakpoint close to position X:21637932. Sequencing of the amplified fragments showed a complex rearrangement in the Rev line, resulting in a large deletion (X:21638367..21684449) containing ZAM, Idefix, Phidippo, and Pifo (sequence in GenBank accession nos. KF410639–KF410642). It must be noted that this deletion could be longer in its proximal part, but it was not possible to analyze it further due to the available sequence of flam ending in the middle of Pifo at position X:2168449. Nevertheless, we were able to extend the analysis to the entire Pifo element by amplifying a whole element, and part of the Phidippo-LTR in which it is inserted, by PCR in Iso1A and to sequence it (sequence in GenBank accession no. KF364665). Because the large deletion in Rev encompasses the ZAM and Idefix elements completely, these results reveal a strict correlation between the presence of ZAM and Idefix within the flam locus and their silencing in Iso1A and w110r6 strains and, inversely, between their absence from the locus and their derepression in Rev. During the in silico analysis of the flam locus, we found that a 30-kb region in the proximal part is very similar to a region in piRNA cluster_17 (6). Cluster_17 is in an unmapped scaffold (positions U:964336..1041768, with “U” meaning “unmapped”). The similar regions are X:21643521..21673908 in flam and U:1003370..1028994 in cluster_17. Our analysis suggests that part of cluster_17 is a segmental duplication of the flam region. The main differences between the two regions are the following. In cluster_17, the ZAM element is reduced to a solo-LTR, the Pifo and Phidippo elements are both absent, and the mdg1-like solo-LTR is replaced by the Phidippo-LTR in which it is inserted. Thus, cluster_17 is a solo-LTR. The differences should be due to recombination between LTRs for ZAM and to Pifo and Phidippo insertions into flam that occurred after duplication of the region. The sequence identity of the two regions ranges from 96–98%. Like flam, cluster_17 produces piRNAs in follicle cells and in their derived ovary somatic sheath cells (12, 23), suggesting that it could be involved in ZAM and Idefix silencing by production of corresponding piRNAs. Thus, it was important to search for any structural modifications of cluster_17 in Rev, where this silencing is lost. PCR amplifications with primer pairs within region U:1010113..1029656, including all ZAM and Idefix sequences, and in region U:988011..988850 failed to amplify fragments in w110r6 and Rev. These results indicate that the ZAM and Idefix sequences are absent from cluster_17 in w110r6 and Rev. PCR amplifications further revealed that this deletion is larger in Rev than in w110r6 because a region proximal to Idefix (U:1003864..1004568) is present in w110r6 (Fig. 2; PCR results are provided in Table S4). In the Rev strain, we observed a deletion of the proximal flam region and also a deletion within cluster_17. Thus, we hypothesized that cluster_17 might be close to the flam cluster. To test this hypothesis, we used the Df(1)DCB1-35b line (24) containing a large deficiency of the flam locus and surrounding regions (covering regions 19F1-h26) to perform a genetic complementation test as in the study by Desset et al. (9). The Df(1)DCB1-35b line was crossed with the w110r6 or the Rev line, and with Iso1A for control, to obtain females that are heterozygous for the 19F1-h26 deficiency and the flam allele from respective strains. These females present the same phenotype for ZAM and Idefix silencing as the parent Iso1A, w110r6, or Rev line (9). Genomic DNA from these females was extracted and tested for the presence of the different regions of cluster_17 as before. These PCR experiments gave the same results as for the Iso1A, w110r6, and Rev strains above (Fig. S2). Actually, all regions of cluster_17 that gave negative PCR results in the w110r6 or Rev line also gave negative results in the hybrid progeny from crosses with the Df(1)DCB1-35b line, indicating that the deficiency cannot complement the w110r6 or Rev line for cluster_17. These results suggest that cluster_17 is contained in the 19F1-h26 deficiency, thus close to flam, and might be localized in the 75-kb nonsequenced region that is proximal to flam (Fig. 2).
data are consistent with a recent origin from horizontal transfers that occurred between species belonging to the *melanogaster* subgroup.

Bartolome et al. (20) suggested that one-third of the TE families originated from recent horizontal transfer between *D. melanogaster, D. simulans*, and *D. yakuba*. Our data reveal that this rate is even higher in *flam*, reaching two-thirds of the recent elements. This suggests that *flam* is a trap for TEs undergoing horizontal transfer.

**Concluding Remarks: piRNA Clusters and Adaptation to TE Invasions.**

The piRNA pathway plays a crucial role in TE silencing and is conserved among species. However, the mechanism by which this system adapts to new mobile elements is still obscure. Our data show a high insertion rate of recent TEs in the *flam* piRNA cluster far exceeding that previously suspected. Interestingly, Vermaak et al. (27, 28) developed a model in which Rhino protein might interact with the integration machinery of TEs to direct their integration into heterochromatin and, more specifically, into piRNA clusters. Our results concerning *ZAM* and *Idefix* highlight how the presence or absence of retrotransposons in piRNA-producing loci makes some *Drosophila* lines more susceptible to TE invasions than others, and thus how piRNA clusters affect the genomic TE distribution. We observed a strict correlation between the presence of *ZAM* and *Idefix* in the *flam* locus and their silencing. Consistently, their deletion from the *flam* locus observed in the Rev line is correlated with their activation, characterized by high mobilization, instability, and copy accumulation (9, 14). A deeper analysis of the *flam* structure revealed that deletions occur frequently in the locus. Mostly, they affect internal segments of TEs, ranging from several base pairs up to several kilobases, affecting both ancient TEs, remaining as vestiges in the locus, and recently inserted TEs. The longest internal deletions affecting retrotransposons are due to homologous recombination between LTRs leading to the complete elimination of internal sequences. Moreover, large deletions may eliminate several TEs within one mutational event, as seen for *ZAM* and *Idefix* in the Rev line. At the same time, insertions also occur within the *flam* locus, as exemplified by the high proportion of recently inserted TEs, the recent insertions of 412 and Stalk2 in the Iso1A strain, and short and long segmental duplications. Such genetic dynamics of a piRNA master locus open the way to a constant emergence of new patterns of piRNA biogenesis potentially leading to changes in the level of transposition control.

The present data fit well with a model of TE invasion and its subsequent control by the invaded species as follows (Fig. 3). The best genetic background for a TE to invade a genome and have full activity should be a “virgin” genome devoid of any related copy. The best chance to find a virgin genome is to invade another species by horizontal transfer (29). In this genome, the incoming TE is not silenced, and is thus able to transpose at high frequency. A period of instability of the newly acquired TE results in its increased copy number. Insertions into piRNA clusters like *flam* are then highly probable because our data evidence high content of recent TEs in such loci. These insertions would be associated with production of corresponding piRNAs and silencing of homologous elements. Thus, as soon as one copy of the TE is inserted in a piRNA cluster, a period of stability follows. This suggests that TEs regulated by a certain piRNA cluster should be present only once in this locus, as is seen for most TEs within *flam*. One or several deletion events can then lead to elimination of TE copies from the locus. A new period of activity of the remaining functional elements in the genome starts. Because deletion events may delete several elements from the locus, transposition bursts may happen involving several different TEs at the same time. This new period of instability for the TEs offers the opportunity to insert into a piRNA cluster again. When this occurs, stability is regained. Thus, transposition bursts, periods of stability, and periods of instability shaping the *Drosophila* genome would be directly correlated to the mutational events that affect piRNA clusters like *flam*. This scenario supports the hypothesis proposed by Le Rouzic and Capy (30) that successful invasion of a population by TEs should be possible “thanks to an initial transposition burst followed by a strong limitation of their activity.”
Rounds of high transposition rate can trigger genetic instabilities and disease-associated mutations, but there is no doubt that they also play an essential role in the evolution of species. Actually, the current *Drosophila* genome witnesses multiple transposition bursts over time for most of the TE families, resulting in ancient and recent copies being present in the genome (examples from this study are *Blood*, *Stalker2*, *Stalker4*, *Gypsy1*, and *Phidippus*). The case of *Pifo* depicted here is different and certainly represents a case of a new invasion of *D. melanogaster*, because no ancient *Pifo* elements can be found in the genome. Such high dynamism of piRNA clusters should also remodel heterochromatic regions in other *Drosophila* species. In *D. erecta* and *D. yakuba*, *flam* loci have been shown to contain a large amount of TEs that are completely different from the *D. melanogaster* *flam* elements (12). These data illustrate the dynamics of piRNA clusters and their coevolution with the rest of the genome regarding TE content. They also highlight the essential role that piRNA clusters might play in speciation by remodeling via TE control of large genomic regions.

**Materials and Methods**

**Bioinformatics.** Sequences and genomic positions were from Flybase (http://flybase.org) *Drosophila melanogaster* genome release 5.1. The TE content of the *flam* locus was first analyzed by CENSOR using Repbase (www.girinst.org) (18, 19). Elements that had more than 98% sequence identity with the library (or with newly identified TEs) were defined as recent TEs. The sequences having less than 98% sequence identity with the library were considered as ancient TEs. TE annotations were from Flybase (*Drosophila melanogaster* genome release 5.1).