Experimental evolution reveals hyperparasitic interactions among transposable elements

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Transposable elements (TEs) are repeated DNA sequences that can constitute a substantial part of genomes. Studying TEs’ activity, interactions, and accumulation dynamics is thus of major interest to understand genome evolution. Here, we describe the transposition dynamics of cut-and-paste mariner elements during experimental (short- and longer-term) evolution in Drosophila melanogaster. Flies with autonomous and nonautonomous mariner copies were introduced in populations containing no active mariner, and TE accumulation was tracked by quantitative PCR for up to 100 generations. Our results demonstrate that (i) active mariner elements are highly invasive and characterized by an elevated transposition rate, confirming their capacity to spread in populations, as predicted by the “selfish-DNA” mechanism; (ii) nonautonomous copies act as parasites of autonomous mariner elements by hijacking the transposition machinery produced by active mariner, which can be considered as a case of hyperparasitism; (iii) this behavior resulted in a failure of active copies to amplify which systematically drove the whole family to extinction in less than 100 generations. This study nicely illustrates how the presence of transposition-competitive variants can deeply impair TE dynamics and gives clues to the extraordinary diversity of TE evolutionary histories observed in genomes.

Transposable elements | hyperparasitism | Drosophila | experimental evolution | invasion dynamics

The evolutionary factors explaining the distribution of transposable elements (TEs) across organisms are still poorly understood (1). TEs are mobile DNA sequences able to invade populations and to duplicate within genomes by various molecular mechanisms (2) and can be found in multiple copies in virtually all living species. However, the nature and abundance of TEs vary substantially throughout the tree of life (3). Although most prokaryotes harbor only a few insertion sequences, large eukaryotic genomes (including plants, amoeba, or animals) may contain up to 80% of TE-derived sequences.

TEs are often considered as selfish-DNA sequences, meaning that they have a greater chance of being transmitted to the progeny than nonselfish sequences (4, 5). In this hypothesis, the ubiquitous presence of TEs can be satisfactorily explained without adaptationist hypotheses (6). The underlying driving mechanism is replicative transposition, which has two combined consequences: (i) an inflation of copy number per genome over time; and then, in sexual populations, (ii) a tendency of TE copies to be transmitted to the progeny more efficiently than Mendelian factors. Replicative transposition theoretically allows the invasion of populations from a single individual, despite establishment of efficient host regulation; natural selection against deleterious insertions and high TE load; or transposition-related or -unrelated recombination, excision, and deletion (7–9).

Of particular interest is that TE copies from the same family, although derived from a common ancestor, do not necessarily cooperate (10). Whatever the molecular mechanism (e.g., copy-and-paste or cut-and-paste), transposition requires the production of one or several proteins encoded by the TE itself (11). These proteins may promote the amplification of any similar copies, including those that do not produce any functional transposition machinery. Such nonautonomous copies may thus proliferate, provided that at least one active copy is present in the genome. Nonautonomous copies are often very successful and can even out-compete autonomous copies (12, 13). Because both autonomous and nonautonomous copies compete for the same transposition machinery, it is tempting to speculate that the invasion of autonomous copies may be slowed by the presence of nonautonomous copies. Theoretical models have confirmed that such competition could alter considerably the evolutionary dynamics (14–18), and the presence of nonautonomous competitors may be a major explanatory factor for the fact that a given TE may be extremely successful in some species whereas performing poorly in others.

Interestingly, despite its theoretical relevance to understanding genome evolution, there is very little direct experimental support for such a negative interaction between autonomous and nonautonomous copies. The original cut-and-paste mariner transposon, identified first in Drosophila, appears as a good model to experimentally test this assumption. Indeed, two distinct mariner sequences have been isolated from Drosophila mauritiana, a sister species of Drosophila melanogaster (19, 20). Both copies are full-length, but one (peach) is nonautonomous, unable to promote its own transposition due to nonsynonymous substitutions, whereas the other (Mos1) is an autonomous copy able to cross-mobilize peach copies. D. melanogaster does not naturally carry Mos1-peach-related elements, but transgenic lines have been obtained with each of these copies.

Here, we used the mariner system in D. melanogaster through two series of experiments to study the capacity of Mos1-active

Significance

Transposable elements (TEs) are DNA sequences that colonize every genome and have a great impact on the genome evolution and structure. Here, we report experimental evolution results that confirm the intrinsic “selfish” properties of TEs in sexual populations. We also show how different kinds of copies from the same family strongly interfere: cheating nonautonomous copies parasitize autonomous ones, to the extent of endangering the survival of the whole TE family. These results nicely illustrate the “genome-ecology” analogy, according to which genome components are assimilated with interacting species in an ecosystem.

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copies to invade the genome of *D. melanogaster* and to decipher the dynamic properties and evolutionary interactions between nonautonomous and autonomous elements. In both experimental setups, we introduced a single “migrant” carrying a few *Mos1* copies among flies deprived of active *mariner* elements but that may contain an inactive peach copy. First, we checked the ability of *Mos1* to invade empty populations, free of any kind of *mariner*, and quantified the selfish-DNA properties of this element from several hundred independent experiments, by computing the frequency at which TEs were still present after 10 generations (thereafter, “invasion frequencies” experiments). In the second set of experiments (“experimental evolution”), only a few migration events were reproduced. We either introduced migrant with active *Mos1* only in populations with no *mariner* at all or migrants containing both active and inactive copies in recipient populations containing only one inactive copy. We tracked the number of copies through genomic quantitative (q)PCRs for both *peach* and *Mos1* elements, independently, and when feasible, we followed the dynamics of the invasion process through phenotypic markers, for up to 100 generations.

**Results**

**Invasion Frequencies.** We initialized a total of 272 invasion experiments in which a *Mos1*-carrying migrant from two strains (male or nonvirgin female) was introduced into small populations of 9 flies without any *mariner* TEs (*SI Appendix, Supplementary Methods*). Experiments were maintained up to 10 generations. *Mos1* copy number was estimated by qPCR on the migrant, and the presence of *Mos1* was assayed by PCR in the progeny at generation (G)5 and G10 for each experiment separately. For statistical analysis, we only kept experiments in which the migrant copy number was between one and five (i.e., 47% of the initial experiments). There was a significant departure from the expected distribution of copy numbers, both for the mean (around seven copies in average, whereas only five were expected) and for the shape [a significant departure from the theoretical Poisson distribution (21); *SI Appendix, Supplementary Results*]. This result can be explained by replicative transposition during the initial crosses. We also removed experiments with poorly replicable PCRs or inconsistent scenarios [e.g., absence at G5 and presence at G10]. This procedure resulted in 94 experimental lines, representing 34% of the initiated experiments. On average, the *Mos1* element was maintained among 71% of these populations at G10 (Fig. 1; for more details, see *SI Appendix, Supplementary Methods and Supplementary Results*).

We used a binomial generalized linear model (GLM) to analyze invasion frequencies at G10 and observed a strong and significant sex effect (invasion frequency higher in females than in males) (*P* = 0.002), as well as an effect of the migrant copy number (*P* = 0.024). The strain factor was not significant, and the data from the two strains were pooled for further analysis. We compared the invasion frequency of *Mos1* elements with theoretical expectations in absence of transposition, calculated through simulations (see Fig. 1 and *SI Appendix, Supplementary Results*), with two population sizes (*N* = 10 and *N* = 20). Both a binomial test and an exact distribution test using a Monte Carlo approach revealed that *Mos1* invaded populations more frequently than predicted in absence of transposition, even in the more conservative scenario (*N* = 20, two-tailed test, *P* = 0.03 for males and *P* = 0.001 for females).

The same analysis at G5 leads to the same trends but lacks statistical power (*SI Appendix, Supplementary Results*) due to the lower frequency of theoretical loss by drift. The data confirm that *Mos1* is well suited to invade *D. melanogaster* naive populations, even when the initial copy number is low. The major factor conditioning the invasion success is the sex of the migrant, because females are very likely to reproduce, whereas the mating success of males is more stochastic. Nevertheless, even a low-copy number male migrant still has a >40% probability to trigger a successful TE invasion in the population (Fig. 1), illustrating the selfish-DNA efficiency of *Mos1* elements.

**Experimental Evolution.** Transposition is expected to increase both the probability of invasion (as evidenced in the invasion experiments) and the genomic copy number in each individual of the population. We thus ran long-term evolutionary experiments to track the dynamics of the average copy number, initiating populations with two types of copies (autonomous and nonautonomous) in migrants of different sexes (*SI Appendix, Tables S1 and S2*). For discriminating both types of copies (autonomous vs. nonautonomous elements) in the same population, we developed an efficient methodology based on qPCR. In parallel, we also followed the frequency of *Mos1* carriers during the first generations using a phenotypic assay (*SI Appendix, Supplementary Methods*).

**Autonomous elements.** We first monitored the amplification dynamics of *Mos1* elements alone in *Mos1*-free *D. melanogaster* populations. To increase the chance of successful invasions, we introduced only single wild-type *Mos1*-carrying nonvirgin females (the most favorable scenario for TE invasion) in different *mariner*-free strains (*yellow white* (yw) populations (experiment A1) or wild-type populations (experiment A2)), with the hope of detecting the potential influence of the genetic background and/or phenotypic markers. As a control, we ran experiment A3 initialized with 100 initial TE carriers, to study the amplification dynamics in a population already contains TEs. A1 and A2 migrants and A3 flies all resulted from successive backcrosses of the M19 strain with the A2 recipient strain.

All dynamics displayed a similar pattern, with a continuous increase in copy number for 50 generations (Fig. 2 A–C). There were significant differences between experiments [analysis of covariance (ANCOVA); *P* = 10⁻⁴] but not between replicates of the same experiment (*P* = 0.87). From G10, we observed less *Mos1* copies in A1 than in A2 (differences in the intercept in a linear model; *t* test: *P* = 0.012) or A3 (*P* < 0.001). At G50, there were about 10 copies per haploid genome in A1 vs. about twice as much (20 per haploid genome) in A2 and A3 (Fig. 2 A–C). Furthermore, transposition rates calculated from a linear regression of log(copy number) over generations were lower for experiment A1 [0.013 transpositions per copy per generation; 95% confidence interval (CI): 0.006–0.021] than for experiment A2 (0.023; 95% CI: 0.018–0.028) or A3 (0.022; 95% CI: 0.020–0.029), the difference between A1 and A3 being statistically supported (*t* test: *P* = 0.004). More details are available in *SI Appendix, Supplementary Results*. 

![Fig. 1.](https://www.pnas.org/cgi/doi/10.1073/pnas.1524143113)
For all experiments, the invasion dynamics of the Mos1 element was fundamentally altered in presence of nonautonomous peach copies. The amplification of Mos1 stopped rapidly (after less than 10 generations), and the copy number stabilized around 3 copies per haploid genome. Furthermore, the number of Mos1 elements tended to stabilize or even decrease (Fig. 3A), and Mos1 elements were virtually lost in all time series by G100 (less than one copy per haploid genome in experiments ANA1 and ANA2 and undetectable in all three ANA3 time series). The absence of active Mos1 was confirmed for all experiments at G120 to G130 by phenotypic test crosses (SI Appendix, Supplementary Methods).

Conversely, nonautonomous peach copies amplified dramatically, from 1 copy at G0 to 15–30 copies per haploid genome by G60. After G60, the peach copy number stabilized, which is likely due to the loss of the source of transposase from Mos1 (segmented regression: breakpoint at G62.4 ± 5.1; SI Appendix, Supplementary Results). Between G10 and G60, transposition rates were 0.040 (95% CI: 0.021–0.059) per peach copy per generation in experiment ANA1, 0.039 (95% CI: 0.033–0.045) in experiment ANA2 and 0.014 (95% CI: 0.008–0.021) in experiment ANA3 (Fig. 3B). The latter was significantly different from the first ones (t test from a linear model: P < 0.001). Thus, introducing more initial copies (in ANA3) might have impaired the invasion success of autonomous and nonautonomous copies (both in terms of transposition rate and final genomic TE content).

The major pattern emerging from experimental evolution is that actively transposing elements are Mos1 copies when alone and peach copies when both autonomous and nonautonomous elements are introduced. Indeed, both standalone Mos1 and nonautonomous peach transpose with approximately the same rate (around 0.02 duplication event per copy and per generation) (Fig. 3B). A one-way ANOVA considering three groups (ANA/Mos1, ANApeach, and A/Mos1) highlighted significant differences in transposition rates between Mos1 and peach within ANA (posthoc Tukey test, P < 0.001) and between ANA/Mos1 and A/Mos1 (P < 0.001) but no differences between ANApeach and A/Mos1 (P = 0.84). Interestingly, Mos1 elements when peach are present display a negative transposition rate (i.e., they are more often deleted than amplified) (ANA1: −0.010; ANA2: −0.001; ANA3: −0.034; the negative rate being statistically significant for both ANA1 and ANA3; SI Appendix, Supplementary Results).

This result demonstrates the strong interaction between autonomous and nonautonomous copies, because autonomous Mos1 elements stopped transposing in the presence of nonautonomous peach elements.

**Early invasion.** During the first generations, the estimation of transposition rate is complicated by the fact that some flies do not contain the autonomous element. Indeed, the rise in average copy number of mariner elements involves both the increase in copy number within TE-carrier individuals and the increase in frequency of TE carriers. However, we could disentangle both phenomena in ANA experiments, taking advantage of the phenotypic effect of Mos1-triggered excision of the peach copy, to distinguish between TE-carrier flies and -noncarrier flies (SI Appendix, Fig. S2). Indeed, in the presence of Mos1, peach, originally inserted into the white gene, excises, which restores the gene activity. Hence, excision is easily visualized by the eye color, and this system can be used as a phenotypic assay for testing transposition activity (20). Hence, we could estimate both the average copy number in Mos1 carriers and their frequency in the population and thus estimate the real transposition rate among Mos1 carriers. Fig. 3C shows the copy number of Mos1 and peach copies among Mos1-containing flies, as well as theoretical predictions under the hypothesis that there is no transposition, no selection, and assuming random mating (see SI Appendix, Supplementary Methods for more details). The discrepancy between

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**Fig. 2.** (A–C) Copy number dynamics of the Mos1 copies when the autonomous element is alone. Bars represent SEs estimated from the qPCR analysis. Graphs differentiate recipient populations with different genetic backgrounds and overlapping curves indicate independent replicates. (A) [yw] population with no Mos1 copy. (B) [y" w"] population with no Mos1 copy. (C) [y" w"] population containing Mos1 copies. (D–F) Copy number dynamics when the migrant brings both autonomous Mos1 (red) copies and nonautonomous peach (orange) copies. Recipient populations contained one peach copy per genome. Graphs correspond to different migrant categories. (D) Population initiated with one male. (E) Population initiated with one virgin female. (F) Populations initiated with one nonvirgin female.

**Nonautonomous along with autonomous elements.** We initialized 11 populations, all containing 1 inactive peach copy, whereas migrants consisted in 1 male (ANA1), 1 virgin female (ANA2), or 1 nonvirgin female (ANA3), all carrying few copies of both Mos1 and peach. In such conditions, Mos1 carriers were easily detected by their eye color (SI Appendix, Supplementary Methods and Fig. S2), and we could thus readily monitor the invasion success for different kinds of migrants. The invasion of Mos1 was successful in one replicate of three for experiment ANA1, one of five for experiment ANA2, and three of three for experiment ANA3 (Fig. 2 D–F), confirming that starting with one nonvirgin female as a migrant is the most favorable condition. In the same way, for the autonomous copies alone, there was a significant effect of the experiment for Mos1 (ANCOVA, \( P = 10^{-5} \)) but not for peach (\( P = 0.43 \)), and the experimental evolution was highly replicable (no effect of the replicate; \( P = 0.59 \) for Mos1; \( P = 0.43 \) for peach; SI Appendix, Supplementary Results).
observed and theoretical copy numbers suggest a substantial rate of replicative transposition for both Mos1 and peach. Average transposition rates calculated on these first generations were, for Mos1, 0.45 per copy and per generation in experiment ANA1 and 0.33 in experiments ANA2 and ANA3. For peach, these rates were respectively 0.75, 0.53, and 0.49 for ANA1, ANA2, and ANA3. Therefore, transposition rates in the very first generations of the invasion were at least one order of magnitude larger than their average over long-term experiments. We also checked that natural selection was not responsible for the seemingly elevated transposition rates. Even strong selection (s = 0.5) against Mos1-free individuals had a modest impact on transposition rate estimates (25% decrease in the transposition rate of Mos1 and 7% increase in the transposition rate of peach). Hence, the hypothetical effect of natural selection is unlikely to affect our conclusions qualitatively.

Discussion

Our experimental results confirm and expand theoretical expectations on transposable element dynamics. First, we showed that active mariner elements behave exactly as expected under the selfish-DNA hypothesis: active transposition promotes both the invasion of the population (the frequency of TE carriers in the population increases deterministically) and the colonization of the genome (the number of copies per individual increases with time). Second, our results demonstrate a strong dynamical interaction between different kinds of copies (autonomous vs. nonautonomous), leading to specific evolutionary patterns depending on the presence of nonautonomous copies, which appear to efficiently act as parasites on autonomous elements.

Experimental Design. Testing the selfish-DNA hypothesis consisted of verifying that TEs are able to invade populations better than expected by drift only. We chose to obtain invasion frequencies under conditions (similar genetic backgrounds between migrant and recipient, two different starting strains, two independent replicates), allowing to rule out any confounding drive effect due to alleles that could be present in the migrant strain. Furthermore, indeed we did not detect any effect of the strains or of the replicates. However, this experimental design prevented us to use any neutral markers for estimating the drift force, and we compared the observed frequencies to simulations with arbitrary population sizes ($N_e = 10$ and $N_e = 20$). Although vials may contain from a dozen to a hundred flies, these figures correspond to conservative assumptions for effective population sizes: simulated values assume a ratio $N_e/N$ of about 0.1–0.2, which remains above empirical estimates in Drosophila (22, 23).

The strong effect of the migrant sex on invasion frequency is consistent with the theoretical difference obtained in simulations, due to the combined effect of (i) the fact that migrant females are already fertilized by TE-carrying males and (ii) the assumption that all females can lay eggs, whereas some males are excluded from the reproduction. Our experimental design makes it impossible to exclude the (likely) hypothesis of a different transposition rate in males vs. females. Furthermore, our results suggest that the number of copies carried by the migrant might be less important for females than males (Fig. 1), but the sex × copy interaction failed to reach statistical significance in the GLM analysis ($P = 0.058$).

In experimental evolution with competition, TE-invasion tracking was facilitated by phenotypic markers indicating the presence of active TEs in individuals. Although convenient, similar genetic systems have already been suspected to bias the results because TEs might also be driven in populations due to natural selection on marker phenotypes (24, 25). However, we deem it unlikely that the observed patterns could be explained by spurious selection: (i) in our system, phenotypic markers are not within the elements, and can then be easily decoupled from TE dynamics within a few generations due to sexual reproduction and recombination; (ii) the amplification dynamics and copy number in yw populations were never higher than in wild-type populations; and (iii) including selection in the formulas used to estimate short-term transposition rates shows that selection has a moderate effect on transposition rate estimates. Consequently, even if we cannot formally exclude a minor quantitative effect of selection, especially in the very first generations, we are confident that the observed dynamics are mainly driven by transposition.

Consistency with Existing Experimental Knowledge and Generalization. TE-invasion experiments in eukaryotes have already been carried out from active copies introduced by transformation, most of the time in D. melanogaster or close species (26). To our knowledge, few experiments have been designed to allow TEs to invade freely an empty population (e.g., ref. 27), and no experimental study has focused on the invasion frequency. If TE interactions have already been studied at the functional level (28, 29),
describing the interacting dynamics of several TEs sharing the same transposition machinery at the population level is a unique feature of our experimental design. Overall, the general pattern of active TE invasion is consistent across experiments. P elements from recent natural populations introduced into old laboratory populations of D. melanogaster tended to multiply up to 50 copies per genome (30), whereas the hobo element seemed to stay under 20 copies per genome (31). A decrease in the transposition rate with time is not necessarily observed; for instance, the roo retrotransponson has been shown to be able to accumulate more than 80 copies per genome in mutation-accumulation experiments and even more in specific genetic backgrounds (32). Our results suggest that the upper limit for mariner is around 30 copies.

Average transposition rates rarely exceed $10^{-3}$ events per copy and per generation when measured in natural populations (33, 34). Here, we observed replicative transposition rates ranging from 0.3–0.5 per copy and per generation during the very early stages of the invasion and 0.01–0.03 for the 50 subsequent generations. These figures are of the same order of magnitude as for active P elements during hybrid-dysgenesis stages recorded in the laboratory (35), although dysgenic symptoms were never observed for mariner.

The need to focus on a specific experimental setup, and, in particular, on a specific species–TE pair, necessarily raises issues related to the generality of the results. Here, the choice of D. melanogaster was driven by the facility of transformation and genetic manipulations in this model species. D. melanogaster is also known to be susceptible to TE invasion in the wild (36), and three new TEs have very recently (i.e., during historical times) colonized its genome: the P element (37), the hobo element (38), and the I element (39). It has also been shown experimentally that D. melanogaster’s P element was more efficient than in its sister species Drosophila simulans (40). The recent discovery of P element in natural populations of D. simulans (41) might help to confirm the effect of the host species on TE dynamics in the wild. In addition, Mos1 is known to be an extremely active copy in D. melanogaster (42). In sum, the observed success of the experimental invasions might overestimate the activity compared with an average TE colonization in the wild. However, because observed transposition rates and final genomic copy numbers remain standard for laboratory studies in this species, our results are unlikely to be particularly unrealistic.

Transposition Regulation. In the experimental evolution experiments, we observed a high initial transposition rate during the first generations, followed by a systematic decrease. Rapid changes in transposition rates have also been observed for P elements and suggest the involvement of transposition regulation mechanisms. Two types of autoregulation (by copy number or transposase type) have been previously suspected for Mos1, based on genetic studies in Drosophila. The first is called overproduction inhibition [i.e., formation of inactive aggregates of the transposition machinery when too much transposase is produced (43)]. Although in vitro, cellular, or biochemical studies demonstrated an influence of MOS1 concentration on its cellular localization, and the synaptic complex formation, an effect on transposition rate was never observed (44–46). The second mechanism is dominant-negative complementation (43, 47) between peach and Mos1 that could occur in competition experiments only. Indeed, the peach copy (differing from Mos1 by 11 SNPs) is probably transcribed and translated like Mos1, generating inactive transposase monomers. With a large amount of peach copies, most active MOS1 monomers could be trapped into inactive dimers, decreasing the transposition efficiency.

Drosophila TEs are also known to be host-regulated by the PIWI pathway, mainly through maternal transmission of cytoplasmic small RNAs [PIWI-interacting (pi)RNAs] able to silence TEs on a sequence-specific basis. As seen for the P element-triggered hybrid-dysgenesis syndrome, progeny lacking the silencing maternal transmitted piRNAs displays high transposition rates of the father-transmitted TE and are characterized, for P elements at least, by various mutational defects (sterility, lethality, and developmental problems) (48). The silencing piRNAs in nondysgenic progeny emanate from the transcription of maternal genomic pi-clusters containing TE copies (9). pi-clusters containing Mos1 could be present in our transgenic strain (carrying Mos1 for about 15 y) and then in the migrants (despite several backcrossed against a Mos1-free strain) but not in the recipient population. An elevated transposition rate could then occur in some crosses, before the spread of this hypothetical Mos1-containing pi-cluster. Alternatively, a de novo insertion of Mos1 into a pi-cluster would allow progressive establishment of silencing.

Conclusions

In genomes, some sequences survive by collaborating (such as genes contributing to the survival and reproduction of individuals), whereas others tend to develop conflicts with each other. It has been recently suggested that the relationships between genome components (including genes, transposable elements, or any sequence able to persist over evolutionary time) were similar to the relationships between individuals or species in ecosystems (10, 49, 50), although the possibility to apply ecological formalism to genome evolution remains questionable (51). Here, we brought substantial evidence that the relationships between autonomous and nonautonomous mariner TE copies were analogous to parasitism: Mos1 copies (the “hosts”) are able to survive and replicate by themselves, whereas peach copies (the “parasites”) are unable to transpose without Mos1 copies. When both copies are present in the same habitat (the genome), parasitic copies amplify, which strongly affects the survival and reproduction activity of the host copies. As active transposable elements themselves are often considered as parasites of the genome (52, 53), this genome-ecology analogy would define nonautonomous copies as hyperparasites.

Using Drosophila and mariner as an experimental model, we have been able to demonstrate the strong negative interaction between nonautonomous and autonomous copies of the same family. This interaction reveals a potential weakness of the otherwise efficient selfish strategy of TEs such as mariner, based on self-amplification and spreading through sexual reproduction. The fact that few mutations in a copy can have such dramatic consequence for the TE family gives some explanatory clues to the huge diversity of TE trajectories observed among species. The rapid loss of transposition activity leaves the genome with inactive copies that may stay for a while, be slowly eliminated by drift or by genome deletion, or occasionally reactivated with the arrival of a new active copy. This view is in accordance with genomic data showing that genomes are often riddled with TE remnants. However, this scenario is also counterintuitive because genomes may also contain numerous active TE lineages. With such a rapid inactivation process/loss of activity, the long-term survival of a TE is not uniquely dependent on its selfishness but also on the opportunity to frequently invade new genomes through horizontal transfers, which have been shown to be especially frequent in Drosophila for mariner-like elements (54).

Materials and Methods

Invasion frequencies. Migrant flies containing on average 5 active copies were obtained by 3 successive backcrosses between a Mos1 strain (about 40 copies) and the empty population. Populations were initiated by introducing 1 single male or female fly (migrant) carrying among 9 flies deprived of mariner TEs, keeping an even sex ratio (SI Appendix, Supplementary Methods and Table S1). The migrant, marked by cutting a small piece of wing, was
Experimental Evolution. For the long-term dynamics involving Mos1 only, migrants contained about five Mos1 copies per haploid genome. For experimental assays involving w[111] populations, migrants carried approximately five Mos1 and five peach copies per haploid genome (SI Appendix, Supplementary Methods and Table S1). Invasion dynamics were initialized with 1 migrant individual among 99 recipient flies, in 250-mL bottles raised at 25°C. Flies were allowed to lay eggs for 2 d and then frozen. New emerged flies were collected every 10–12 d later, and 200 progeny flies were used to set up the next generations. Three to five replicates were initialized for all invasion dynamics, although some populations were subsequently lost. qPCR assays were run to quantify the number of Mos1 and peach copies in every generation from G1 to G7 (in 9 Mos1 carriers and empty flies, separately) and in every five generations afterward.

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