Biological Journal of the Linnean Society, 2003, **79**, 33–41.

Intraclonal genetic variation: ecological and evolutionary aspects.
Edited by H. D. Loxdale FLS, FRES and G. Lushai FRES

Transposable elements in clonal lineages: lethal hangover from sex

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Long-term coevolution of transposable elements (TEs) in sexual hosts leads to evolution of extremely active and dangerous mutagens kept in tenuous check by host-derived mechanisms and via natural selection against TE-rich genomes. To the extent that sexual reproduction and recombination are important in maintaining a stable TE copy number and a tolerable mutation load, the switch to clonality from sexual reproduction can be extremely damaging and, generally, should lead to clonal lineage extinction. Surprisingly however, the loss of powerful selective mechanisms constraining TEs can be beneficial in the short-term by immediately eliminating selective load and possibly promoting the early success of clonal lineages. The clonal lineages that do survive in the long-term must find a way to eliminate or domesticate TEs. Indeed bdelloid rotifers, which are ancient asexuals, do appear to have lost most of the otherwise wide-spread TEs and might have domesticated others. The path to this TE-free haven is anything but clear at the moment. We have considered a novel scenario of instantaneous inactivation of TEs by starting off with a genome carrying repressive host alleles for all TEs in the genome. We show that such a scenario appears plausible and provide some limited empirical evidence in its support. *Biological Journal of the Linnean Society*, 2003, **79**, 33–41.

ADDITIONAL KEYWORDS: *Drosophila melanogaster* – ectopic recombination – mutation-selection balance – parthenogenesis – transposition rate variation.

INTRODUCTION

Population dynamics of TEs in natural populations

Transposable elements (TEs) have been found in practically all organisms studied. Across species, as much as 10–90% of the genome is represented by TE-derived sequences, with the human genome closer to the 90% end of the spectrum. Related TE sequences are classified into families, the number of which varies from five in *Saccharomyces cerevisiae*, to 30 in *Candida albicans* (Goodwin & Poulter, 2000) to more than 50 in *Drosophila melanogaster* Meigen (reviewed by Charlesworth, Sniegowski & Stephan, 1994). The number of copies per family also varies from several, as for *gypsy* in *D. melanogaster* (Kim *et al.*, 1990) to hundreds of thousands for *LINE1* in humans (Kazazian & Moran, 1998). Transposable elements have been found to cause up to 50% of visible mutations in *Drosophila* (Finnegan, 1992a,b), cause a wide spectrum of chromosomal rearrangements (see for example, Cáceres *et al.*, 1999), including some responsible for known human diseases (Reiter *et al.*, 1996). Given the abundance of TEs and the profound effects that they have on the genome structure, content and function, it is essential to understand the evolutionary forces responsible for the persistence and diversification of TEs.

TEs multiply by making paralogous copies of themselves in the genome. The ability to multiply faster than the non-transposable genes allows for TE persistence despite many adverse effects on the host performance. These adverse effects must ultimately 'contain' the potentially unlimited multiplication and the ever-increasing representation of TEs in the genome (Charlesworth & Charlesworth, 1983). Such containment must either come about through regulation of transposition via TE-encoded and host-driven mecha-

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nisms (as copy number increases, rate of transposition decreases), or through direct selection against genomes with higher copy numbers of TEs. These possibilities are not mutually exclusive. Indeed in *Drosophila*, where most of the research has been conducted, we have clear evidence of both regulation of transposition and selection against TEs within populations.

For the sake of simplicity, let us first consider a case of unregulated transposition. A few conditions should hold for natural selection to prevent TE explosion. Firstly, fitness must decline very fast with TE copy number (logarithm of fitness should decline faster than linear) to compensate for inherently exponential multiplication of TEs (Charlesworth & Charlesworth, 1983). If fitness decreases slower than that, TEs will proliferate, leading to continuous reduction in organismal fitness and most likely to eventual population extinction, as was first observed by Preston & Engels (1989). Secondly, the populations should be sufficiently large for individual TE inserts to have very little chance of reaching population fixation. In small populations, the variance in TE copy number is reduced, challenging the efficacy of natural selection that controls TE copy number (Brookfield & Badge, 1997). Depending on TE fitness costs, the resulting expansion of TEs will either pose little danger to the organism, merely increasing the amount of junk DNA, or it may prove fatal.

When selection does maintain TEs at a steady level, theoretical models predict moderate, although noticeable, reduction in fitness. Given the estimates of transposition rates measured in populations (Nuzhdin & Mackay, 1995), fitness of TE-containing hosts should be reduced by 0.5–5% relative to the TE-free hosts (Charlesworth & Langley, 1989). This means that on the one hand, natural selection can maintain TEs without suffering overwhelming consequences to fitness. On the other, that the hosts that somehow eliminate fitness reduction due to TEs would gain a substantial selective advantage to spread, even in relatively small populations (i.e. as long as $N_e \gg 20$–200).

Three distinct but not mutually exclusive hypotheses have been proposed for the mechanisms of selection against TEs (for a review, see Nuzhdin, 1999): (i) individual TE copies may be deleterious because they disrupt genes ('gene-disruption model'; Finnegan, 1992a; McDonald *et al*., 1997); (ii) transcription of TEs and translation of TE-encoded proteins may be costly, and these transcripts and proteins may generate deleterious effects by nicking chromosomes and disrupting cellular processes ('TE-product expression model'; Nuzhdin, 1999); and (iii) a high copy number of TEs could be deleterious because ectopic recombination among dispersed and heterozygous TEs generates strongly deleterious chromosome rearrangements ('ectopic recombination model' Montgomery, Charlesworth & Langley, 1987).

It may not be especially controversial to assume that all three of these mechanisms act upon many TEs in natural populations. What is controversial, however, is which of these forces is the primary one limiting the spread of those TEs that segregate in natural populations. For instance, even though TEs do undoubtedly cause deleterious mutations by inserting into genes, it is possible that the majority of such mutations are extremely deleterious. In reasonably large natural populations such mutation will be present at very low population frequencies and will contribute very little to the observed genomic TE-content. TE elements that do segregate in nature should experience selection of the strength 10^{-5}–10^{-4} per copy, with no inserts completely neutral (Charlesworth *et al.*, 1994). It is likely that for the TE insertions into the 'junk' regions of the genome it is either ectopic recombination or TE expression that generate these negative fitness effects

As mentioned above, some TEs may be subject to self-regulation. Theoretical studies have demonstrated that TEs in out-crossing populations should in general evolve to have the highest possible rates of germ-line transposition. Self-regulation is likely to evolve only in the case of abundant selfing and/or nearly absent recombination (or prevalence of short-distance transpositions; Charlesworth & Langley, 1986). This prediction is hard to test, given that even when we observe clear reduction of transposition rates with the copy number (for instance, overproduction inhibition of *mariner* transposition where transposition rate goes down as the concentration of transposase increases; Lohe & Hartl, 1996), it is often unclear whether the effect is simply a side, 'hard-to-avoid' effect of transposition mechanics or the adaptively evolved self-regulation.

While TEs generally experience selection favouring the most active copies, the host genome is selected to repress transposition (Charlesworth & Langley, 1986). However, similar to selection for modifiers of the rate of mutation, this selection is rather weak (Charlesworth & Langley, 1989). Thus, host alleles constraining transposition should accumulate, but not necessarily fix. Frequency of permissive alleles may thus be a key factor defining average transposition rate. Fixation of different host alleles in laboratory isogenic derivatives might be responsible for the transposition rate variation uncovered between laboratory lines of *D. melanogaster* (Nuzhdin, 1999). To take an example of one TE family, many laboratory stocks are known to have actively transposing LTR-Containing retrotransposable element *copia* (Biemont *et al*., 1987; Di Franco *et al*., 1992; Pasyukova & Nuzhdin, 1993; Charlesworth *et al.*, 1994; Nuzhdin &

Mackay, 1994). While we cannot exclude the possibility that these stocks have accumulated new mutations affecting *copia* transposition rate before *copia* instability was found, it is more likely that permissive/restrictive alleles segregate in nature.

The maintenance of permissive/restrictive alleles at intermediate frequencies can be easily envisaged. If the rate of transposition is 10^{-3} per copy in a permissive background and zero in a restrictive background, the selection coefficient for restrictive allele can be calculated as $\sim 2 \times 10^{-4}$ (Nuzhdin *et al*., 1998). Then, if the rate of mutations to permissive allele is 10^{-6} ('typical' per locus mutation rate, Ashburner, 1989), and permissive alleles are recessive (Pelisson *et al*., 1997; Pasyukova, Nuzhdin & Filatov, 1998) the equilibrium frequency of permissive alleles is ~7% (Falconer & Mackay, 1996) fitting well with the average rate of transposition in nature $\sim 10^{-5}$–10^{-4}.

In the above example, the suppression appears to be specific to *copia*. This is also the case in several other examples, such as suppression of LTR-retrotransposable element *gypsy* by a host gene *flamenco* in *D. melanogaster* (Prud'homme *et al*., 1995; Robert *et al*., 2001) and in some cases of hybrid dysgenesis (cases of high rates of TE transposition brought about by some conspecific crosses; Kidwell, Kidwell & Sved, 1977; Bingham, Kidwell & Rubin, 1982; Eggleston, Johnson-Schlitz & Engels, 1988; Finnegan, 1989). In other cases, multiple unrelated TEs can be suppressed by a common mechanism. In one example of hybrid dysgenesis found in *Drosophila virilis* Sturtevant (Lozovskaya, Scheinker & Evgen'ev, 1990), at least five unrelated TEs are simultaneously released from repression in some crosses (Petrov *et al*., 1995; Evgen'ev *et al*., 1997; Vieira *et al*., 1998). Repression of TEs through homology-mediated mechanisms (RNAi-mediated, see review by Plasterk, 2002), may also result in the control of multiple TEs by the same host gene(s) (Aravin *et al*., 2001).

In this article, we critically examine the recent experimental results of authors that have added to our current understanding of TE maintenance in host genomes. At the end, we discuss the possible impacts of harbouring aggressive TEs for the evolution of clonal populations.

THE EXTENT OF FITNESS REDUCTION CAUSED BY TEs

The extent of fitness decrease due to TE accumulation remains a matter of debate. In *Drosophila*, Eanes *et al*. (1988) carried out an experiment to measure the hemizygous fitness effects of *P*-transposon insertions in the *X* chromosomes of males. They regressed fitness on the number of elements and estimated the mean homozygous effect as 1.4% per insert. In contrast, Mackay, Lyman & Jackson (1992) estimated the mean effect on viability of homozygous flies with a *P* element insert into a third chromosome as 13%. This rather large estimate was supported by the data of Lyman *et al*. (1996) who studied *PlArB* insertions. The authors stressed, however, that the effects might be overestimated. This is probable, since *P* elements are likely to make many aborted attempts of transpositions before landing successfully. Insertions might also happen in one cell generation with imprecise excisions later on (the idea is contributed by C. Langley). Thus, the large estimate above might represent a cumulative effect of multiple undetected mutations. The estimates of Eanes *et al*. (1988) are free from this bias since, with their design, effects of undetected mutations enter error variance. Note that the data are limited to one system of induced transpositions of one DNA transposon in a background of a naïve host, previously *P* element free. Whether or not these effects are quantitatively similar to the effects of TEs which have undergone long coevolution with the host remains to be described. A new way of assessing fitness effects of TEs is to measure fitness of laboratory strains of eukaryotic organisms before and after they experience spontaneous accumulation of various TEs, pre-existing in the host. Here we summarize the results of recent experiments in which retrotranposable elements were allowed to accumulate in laboratory strains of *D. melanogaster*.

D. Houle & S. Nuzhdin (unpubl.) accumulated mutations in multiple replicates of an isogenic line of *D. melanogaster* for tens of generations, including those from TE jumps. The line carried permissive alleles in genes normally suppressing *copia* transpositions (Nuzhdin, 1999). Selective elimination of TEs was minimized by using the smallest possible population size of each replicate, one male and one female. This meant that only lethal or semi-lethal insertions of *copia* were 'visible' to selection. The average rate of *copia* retrotranspositions in these replicates was directly measured and was equal to 0.88 per male gamete per generation, or 0.44 averaged between sexes (*copia* does not transpose in females). *copia* copy number, detected by *in situ* hybridization with labelled *copia* DNA, increased at the rate of 0.24 per gamete per generation. Due to the large standard errors for the estimates, this difference was consistent with anything from 'no selection' to 'selective elimination' of three quarters of the inserts. The fitness due to the increase in the *copia* copy number was estimated as the number of offspring produced, relative to a standard genotype. A cryopreserved control had significantly higher fitness than the 'mutation accumulation' lines. The authors found significant among-line genetic variance, arising from novel accumulated mutations. Relative fitness declined by an average of

0.95% per *copia* transposition. Per generation, decline in fitness was 0.34–0.74% with *copia* caused mutations accounting for 0.23%, or approximately one half of fitness decline.

In a similar experiment by Fry *et al*. (1999), mutations were accumulated in chromosomes maintained in heterozygous condition with balancer-chromosomes, which do not recombine with the chromosome serving as the target of TE accumulation (balancer-chromosomes carry a large number of inversions along their lengths which restricts recombination with their homologs; they are often used in *Drosophila* genetics to preserve chromosome integrity). Thus, TEs were shielded from selection due to the three factors: small population size, heterozygous insert state exposing dominant but not recessive deleterious effects, and possible suppression of ectopic exchange (along with recombination). Permissive host genotypes allowed *copia* to transpose, and an average of 2.2 *copia* transpositions per second chromosome per line were accumulated over 33 generations. The effect of *copia* on viability was measured by competing the TE-accumulated chromosome with the balancer-chromosome. The slope of the regression of viability on *copia* copy number showed a mean effect of approximately 2% per insert (Fry & Nuzhdin, in press). The estimated rate of viability decline due to *copia* is 0.14% per generation, while the total rate of viability decline in these lines was previously estimated as 0.24–0.33%.

Lastly, E. Pasyukova, E. Morozova & S. Nuzhdin (unpubl.), accumulated TEs in replicates of the two isogenic *D. melanogaster* laboratory strains 2b and Harwich, with a permissive background for transpositions of *copia*, *Doc*, and *roo* retro elements. The replicates were kept as small laboratory cultures of ten flies, thus selection could have operated/occurred by removing strongly deleterious inserts ($s \sim 0.07$ or more). Here, the authors assessed fitness by measuring the ability of the transposition accumulation replicates to compete against a tester stock. This stock contained a translocation between the second and third chromosomes, resulting in its post-mating reproductive isolation from the tested lines and allowing for a straightforward measurements of relative fitness of the tester vs. tested lines. The authors also estimated the viability of eggs and larvae, both in homozygous stocks and upon mating with an unrelated line. The latter was done to evaluate the effects of ectopic exchange, which is believed to be suppressed between homozygous inserts but not between elements in heterozygous condition (Langley *et al*., 1988). TE accumulation had an effect on all fitness components of the 2b line, with the average deleterious effects of insertion on fitness and its components of ~0.4% for both homozygous and heterozygous inserts.

The results of all three experiments were consistent one with another and showed both the fast increment in TE copy number when unconstrained, as well as the loss of host competitive activity due to TE accumulation. All the experiments were consistent with from a quarter to a half of fitness decline caused by TE mutations. While it has been known that about a half of major morphological mutations in *Drosophila* are caused by TEs (Finnegan, 1992b), the empirical observation of approximately half of moderate effect mutations being induced by TEs is shown for the first time. These laboratory-derived conclusions suggest that TE accumulation should also be deleterious in nature. When is it likely to happen? Brookfield & Badge (1997) argued that TEs are likely to multiply whenever the effective size of a population drops. For instance, selection effectively controlling elements that cause fitness decline of the order of 10^{-5}–10^{-4} in population sizes of 10^5–10^6 will be inefficient in populations of 10^3–10^4 individuals. This is due to two factors. One is that natural selection stops 'seeing' a TE insert if it is accidentally fixed (provided the rate of excisions is low as for most TEs), as should happen frequently in small populations. Another is that deleterious effects caused by different inserts vary, with a substantial proportion of inserts being nearly neutral, and only a few being responsible for most of the fitness decline in the aforementioned experiments. When population size declines, so does the proportion of TEs which affect fitness strongly enough to be effectively acted against by selection (Charlesworth & Langley, 1989). Impressively, it does not take long for TEs in small populations to start growing in numbers: in 500 generations, TEs can theoretically cause complete extinction of some populations (Brookfield & Badge, 1997).

The higher abundance of elements is also predicted in the hosts with higher selfing rates, most likely due to stochastic accumulation. Without excisions, and with recombination strongly reduced by selfing, genotypes with lower element copy number are irreversibly lost as has been shown in extensive simulations (Wright & Schoen, 1999). Wright *et al*. (2001) have recently examined the abundance and levels of insertion polymorphism of an Ac-like transposon family in natural populations of the selfing plant *Arabidopsis thaliana* (L.) and its close out-crossing relative, *Arabidopsis lyrata* (L.) The authors found evidence for recent activity of this element family in both plant species. Transposon 'display analysis' (Waugh *et al*., 1997; Wright *et al*. 2001) showed the presence of slightly higher numbers of insertion sites per individual but fewer total polymorphic insertions in the self-pollinating *A. thaliana* than *A. lyrata*. This is consistent with reduced efficacy of natural selection against TEs in selfing species, and reduction in transposition rate.

The results on the fitness decline upon TE accumulation in laboratory strains are important in showing that all populations, to the extent that they have potentially active TEs, carry in their genome very dangerous agents poised to amplify, reduce fitness, and possibly drive these populations extinct. The long-term coexistence of TEs with their host genomes implies that there must be mechanisms or forces limiting the spread of TEs with major pathways: (i) regulation of TE transposition rate through host-mediated or TE-mediated mechanisms, or (ii) natural selection acting against TEs. Below, we elaborate on one selection mechanism, ectopic recombination between TE copies.

FORCES LIMITING THE SPREAD OF TEs IN POPULATIONS: ECTOPIC RECOMBINATION MODEL

The 'ectopic recombination model' has been vigorously investigated in *Drosophila* (Montgomery *et al.*, 1987; Charlesworth & Langley, 1989). Most studies have attempted to test it by looking at low vs. high-recombination areas of the *Drosophila* genome. To the extent that chromosome regions of low recombination in *Drosophila* also experience reduced rates of ectopic recombination, low recombination areas should exhibit higher abundances and higher population frequencies of TEs. The empirical results have generally met these expectations and could be taken as support for the model (Charlesworth & Langley, 1989). Unfortunately, however, in addition to presumably having a lower rate of ectopic recombination, low-recombination areas also have lower gene densities, likely to permit lower levels of gene expression, and allow for less efficient selection due to the Hill–Robertson effect (Hill & Robertson, 1966). Thus all current selective hypotheses make identical predictions of the higher copy number and higher population frequency of TEs in the areas of low recombination. The fact that this prediction is borne out empirically unfortunately does not readily discriminate between these hypotheses.

In a recent study, D. Petrov's laboratory (unpubl.) has obviated these difficulties by taking advantage of several properties of a particular class of TEs (non-LTR retroelements). Non-LTR elements are attractive as a model system for a number of reasons. Because non-LTR elements do not excise precisely from the genome, excision need not be considered in understanding their population dynamics. In addition, they naturally generate 5′-truncated DOA ('dead-on-arrival') elements as a frequent outcome of transposition (Luan *et al.*, 1993). These DOA elements are not transcribed and do not encode functional proteins. Thus they cannot generate potentially deleterious transcripts and proteins, eliminating selection against deleterious expression of TE-encoded proteins as a possible force acting against individual DOA copies.

The authors concentrated on studying population frequencies of four structurally similar families of non-LTR elements in the *Drosophila* euchromatin. The main finding of this research was that natural selection appears to operate TE family by family, with TEs in some families (*Jockey* and *Doc*) showing signs of very strong purifying selection ($N_e s \ll -1$), whereas others (*BS* and *X*) segregate apparently in a neutral fashion. The variation of selection strength family by family is predicted by ectopic recombination because the strength of selection has to be correlated for all sequences that can recombine with one another (e.g. TEs from the same family). It should be uncorrelated for TEs that cannot recombine with one another (e.g. TEs from different families). On the other hand, it is not predicted by the model of individual deleterious effects of TEs on neighbouring genes. There is no clear reason why TEs from different families should have consistently different effects on neighbouring genes, especially given that the analysed families have very similar structure. In addition, the authors demonstrate that the families do not differ from one another in the distance to the neighbouring genes, with all families containing elements both close and far from genes. Moreover, consistent with the ectopic recombination model, the families showing reduced purifying selection against its elements were less numerous and had shorter copies. This is because the rate of ectopic recombination should be a function of the copy number of TEs within a family and is likely to be an increasing function of their length.

On balance, these results provide evidence that selection against deleterious effects of ectopic recombination could be one force limiting the spread of TEs. It is essential to recognize that these results do not imply that other modes of selection and regulation could not be more important in other organisms and for other types of TEs. They also say nothing about the nature of strong deleterious effects of TE accumulation in lines shielded from selection (see discussion above). Nevertheless, for the discussion of the impact of TEs in clonal lineages, we wished to discuss the evidence that ectopic recombination may be important, at least in some cases.

TEs AND THE EVOLUTION OF CLONAL POPULATIONS

In this section we will discuss the implications of our current understanding of TE evolution and population dynamics for the evolution of clonality. We consider surprising short-term benefits and expected long-term

dangers of inheriting TEs from the sexual past. We also describe how some clonal lineages could avoid being decimated by TEs at the outset.

Sex promotes aggression

In out-crossing sexual organisms, TEs from one genome have an opportunity to transpose to the other genome during meiosis. This allows TEs to be strongly deleterious to their hosts and still spread in populations through continuous transposition. In a seminal paper, D. Hickey (1982) showed that TEs, which can reduce fitness of their host by as much as two-fold, nevertheless still persist in sexual organisms (Hickey, 1982). Not so, however, in the clonal lineages. Except for occasional – and probably exceptionally rare – horizontal transfer among organisms (see Jain *et al.*, this volume), the fate TEs in clonal organisms is entirely linked to that of the host. Long-term, TEs within clonal lineages are expected to become 'nice' and not cause deleterious effects. This is because those clonal lineages that have inactive TEs should in general outcompete those that have active, deleterious ones. Indeed, anciently asexual bdelloid rotifers appear to have lost all of the retrotransposable elements, consistently with such predictions (Archipova & Meselson, 2000).

The problem of course is that asexual lineages generally evolve from within sexual populations or higher-order clades (see Simon *et al*. and Wilson *et al.*, this volume). Suppose that an obligately asexual lineage is established from within a sexual population. Suppose also that this lineage is starting to grow, either by chance or because it has superior fitness, for instance due to chance advantageous combination of interacting host alleles. Because clonal lineages will harbour TEs that have been selected to multiply with little regard for their ancestral, sexual host's fitness, they will continue transposing, in blissful unawareness of the potential suicide that they are therefore committing. To make matters worse, while the lineage is expanding, mutations, including those caused by TEs, are shielded from selection and can accumulate through 'hitchhiking' (Rice & Chippindale, 2001). Such mutation accumulation should generally lead to lethal fitness loss in a population of asexuals (Butcher, 1995).

Short-term advantage from asexuality

Even though it appears that TEs might be more deleterious long-term within clonal lineages, it is important to emphasize that the short-term presence of TEs may provide an advantage for the clonal lineages over their sexual relatives. Imagine again that selection against ectopic recombination is sufficiently strong to contain TEs in the sexual relatives and ancestors. As we discussed earlier, this containment comes at a substantial fitness relative to the genomes free of TEs. and this is exactly the benefit that asexual lineages can accrue simply by turning off all recombination. This advantage should help asexual lineages to flourish short-term; however, long-term it may generally prove disastrous (although see below) if it comes at the expense of allowing TEs to expand uncontrollably in number. Whether or not such a short-term advantage in fact promotes early success of recently derived asexual lineages over their sexual relatives is a matter for further empirical research.

How to avoid dying from asexuality?

One way to avoid the long-term degeneration through uncontrolled transposition would be for transpositions to be directly and mechanistically linked to the abandoned sexuality (Sullender & Crease, 2001). However, most TEs do not obviously depend on sexuality. Alternatively, TEs could die out after accumulating mutations in individual elements. Given that the rate of inactivating mutations in TEs is comparable to the rate of transposition (Nuzhdin *et al*., 1998), it seems likely that TEs will continue to multiply within clonal lineages for a long time until all elements, including the newly transposed ones, are 'dead'. Note that while TEs are dying out, deleterious mutations caused by TEs will still be accumulating; hence clonal lineages would have to survive in competition with their sexual, out-crossing relatives. The result of this competition will depend on many factors, among them the importance of sexual reproduction in eliminating the deleterious mutation load in general (Kondrashov, 1988) and in controlling TE copy numbers. In particular, if ectopic recombination is important in controlling TE spread, clonal lineages should accumulate a higher copy number and suffer greater deleterious consequences from their resident TEs. This is so since the rate of ectopic exchange should be much reduced in selfing hosts due to high homozygosity of inserts (Morgan, 2001), and in parthenogenetic hosts due to reduction of recombination rate (Butlin, 2002). While fitness decreases with TE accumulation, in the absence of ectopic exchange, it may not decrease fast enough to stabilize TE copy number (Wright & Schoen, 1999).

The survival of at least some clonal lineages becomes more plausible when an extra factor is considered. As we mentioned, host factors permitting or suppressing transpositions segregate in natural populations of *D. melanogaster* (Nuzhdin *et al*., 1998). Laboratory lines with permissive alleles undergo TE accumulation, while TEs in the lines with suppressor alleles do not move. We believe that segregation of

suppressor alleles might be key to the establishment of parthenogenetic lineages in other organisms. For instance, if obligate parthenogenesis evolves in a suppressive background, TEs will be stable and the above described process of lengthy TE inactivation 'one-by-one' becomes irrelevant. In a sense, TE stabilization will be achieved instantaneously, not as a result of new mutations that would suppress TE transpositions, but through fortuitously starting off with a TE suppressor allele in the genome.

How likely is it to instantaneously inactivate all of the TEs in the genome by picking a random genomic background? This depends strongly on several factors, most important of which are (i) the number of independently suppressed TE families in the genome, and (ii) the frequency of permissive alleles in the population. For example, if the genome contains 100 independently controlled TE families and the frequency of permissive alleles for each TE is 0.5%, the proportion of genomic backgrounds restrictive for all TE families will be $(1 - 0.995)^{100} = 0.6$. This number, however, goes down to 2×10^{-5} if the frequency of permissive alleles is 10% on average. Unfortunately, we do not know the value for any of these parameters, even in well-studied organisms such as *D. melanogaster*, much less any clonal organism.

Empirical evidence: lengthy inactivation or instantaneous stabilization?

Are currently available data consistent with the hypotheses of lengthy or instantaneous inactivation of TEs? Both hypotheses predict that within a long-established parthenogenetic host population, TEs should be fixed in a set of positions with none or very few recently transposed copies. TE stabilization has indeed taken place in long-ago established asexual lineages of bdelloid rotifers (for elements incapable of horizontal transfer, see Archipova & Meselson, 2000). The TE content was lowered rather than enlarged as the 'lengthy inactivation' hypothesis predicts. Such data cannot, however, be used to reject the hypothesis. Indeed, since TE inactivation happened long ago, secondary substitutions and deletions may have obliterated or even removed the TE-derived portion of the ancestral genome (Petrov, Lozovskaya & Hartl, 1996; Petrov *et al*., 2000).

The instances of relatively recently evolved obligate asexuality might be more informative. Within the genus *Medicago*, the selfing species *Medicago truncatula* Gaertner shows a much lower abundance of '*Bigfoot*' insertions than its close out-crossing relative *Medicago sativa* L. (Cherrier *et al*., 1999). Within *Daphnia pulex* (Leydig) TEs compared between cyclically and obligately parthenogenetic populations do not differ in abundance (Sullender & Crease, 2001). However, while cyclically parthenogenetic populations contain polymorphic TE insertions, each of the surveyed obligately parthenogenetic populations contains only fixed TEs. This is in contrast to non-TE DNA variation which is comparable within out-crossing and selfing populations. Consistently with the instant inactivation model, the copy number of TEs in selfing populations is not increased in comparison with the source outbred population. When independently evolved clonal lineages are compared, the set of fixed TE positions has no more resemblance than between individuals of the source out-breeding population (Sullender & Crease, 2001). All of these features are consistent with multiple independent cases of instantaneous stabilizations.

Predictions to test

We have presented a verbal theory describing short-term advantages and medium-term costs of the transition from sexual to asexual mode of reproduction due to TE multiplication. Interestingly, parasites (and here we think of TEs as genomic parasites) were long considered as one of the agents promoting sexuality, though the costs of asexuality had a different source – lesser genetic variation allowing faster parasite adaptation (Ebert & Hamilton, 1996). While we focused on TEs, the logic similar to one developed above is applicable to any vertically, bi-parentally inherited parasites. Do we envision ways of testing our hypothesis?

Firstly, we need to test whether or not permissive/restrictive alleles for transposition segregate in any organism other than *Drosophila*, preferable in those with facultative parthenogenesis. If segregation is found, we would propose establishing multiple clonal lineages and observing TE dynamics in them. We predict variation between lineages for TE behaviour. Those with stable TEs would have low genetic variation, and little fitness decline over generations. The lineages with active TEs would evolve high genetic variation between congenic replica TEs, and fast overall fitness decline, strongly accelerating over generations. The next experimental step would be to mimic the evolution of asexuality in semi-natural conditions, invoking competition between clonal and sexual lineages.

ACKNOWLEDGEMENTS

We thank Teresa Leonardo, Ben Normark and Olivia Judson for many ideas on evolution of sex and clonality. The research of SVN is supported by NSF DEB-9815621 and NIH 1R01GM61773-01, 1R24GM65513.

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