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The evolutionary life history of *P* transposons: from horizontal invaders to domesticated neogenes

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Abstract *P* elements, a family of DNA transposons, are known as aggressive intruders into the hitherto uninfected gene pool of *Drosophila melanogaster*. Invading through horizontal transmission from an external source they managed to spread rapidly through natural populations within a few decades. Owing to their propensity for rapid propagation within genomes as well as within populations, they are considered as the classic example of selfish DNA, causing havoc in a genomic environment permissive for transpositional activity. Tracing the fate of *P* transposons on an evolutionary scale we describe different stages in their evolutionary life history. Starting from horizontal transfer events, which now appear to be rather a common phenomenon, the initial transpositional burst in the new host is slowed down by the accumulation of defective copies as well as host-directed epigenetic silencing. This leads to the loss of mobility and, finally, to molecular erosion by random mutations. Possible escape routes from genomic extinction are the reactivation within the original host genome by recombination or suspension of the repressing regime, horizontal emigration to a virgin gene pool, or genomic integration and acquisition of a novel function as a domesticated host gene.

Introduction

Over recent decades, transposable elements, which account for a considerable fraction of eukaryotic DNA, have gained acceptance as a major evolutionary force shaping the genomes of their host species as a by-product of their self-propagating lifestyle. One of the most intensively studied examples is the *P* element of *Drosophila*, a family of DNA transposons that has proved useful not only as a genetic tool (e.g., transposon tagging, germline transformation vector), but also as a model system for investigating general features of the evolutionary behavior of mobile DNA (Kidwell 1994). *P* elements were first discovered as the causative agent of hybrid dysgenesis in *Drosophila melanogaster* (Kidwell et al. 1977) and were later characterized as a family of DNA transposons (Bingham et al. 1982). The first sequence of a transpositionally autonomous 2.9 kb *P* element, determined by O'Hare and Rubin (1983), revealed a molecular structure consisting of two terminal inverted repeats of 31 bp, internal inverted repeats of 11 bp, and four exons (numbered 0–3). The exons code for at least two different proteins produced by differential splicing of the primary transcript (Misra and Rio 1990). One protein, the transposase, is made exclusively in germline cells and mediates transposition via a cut-and-paste mechanism and, as recently shown, via "alternative transposition" (Gray 2000). The second protein is translated from an mRNA that still retains the third intron. Owing to a stop codon in intron 3, a shorter protein is formed that acts as a repressor of transposition, inhibiting the genomic mobility of *P* elements in somatic cells (Rio 1990). Transposition also depends on a host-encoded protein named IRBP that binds to the terminal inverted repeats (Rio and Rubin 1988). Since *P* element transposition to a new chromosomal location is, in general, a non-replicative process, a double-strand gap is left behind by the excised copy. This gap is then repaired using the sister chromatid strand as a template (Gloor et al. 1991). Because the gap repair mechanism is relatively error prone, defective copies are often generated in the course of transposition. As long as these defective *P* elements retain intact termini, they are able to transpose passively by means of transposase provided from autonomous copies elsewhere in the genome.

P elements have drawn the attention of evolutionary biologists for two main reasons, namely, their sudden

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appearance in the genome of *D. melanogaster* and their amazing speed of propagation in an infected gene pool. According to historic records (Kidwell 1983; Anxolabéhère et al. 1988), *P* elements started to spread from North America through the natural populations of *D. melanogaster* about 50 years ago and have since extended their range over all continents. This rapid expansion within a few decades, together with the fact that *P* elements are not found in the genomes of the closest relatives of *D. melanogaster* (Brookfield et al. 1984), led to the conclusion that *P* elements must have invaded the *D. melanogaster* gene pool quite recently from an external source. Screening of more distantly related taxa showed that *P* elements are widely distributed in drosophilids. Moreover, the nucleotide sequence of a complete *P* element isolated from the American species *D. willistoni* is almost identical (a single substitution over a length of 2.9 kb) to the *P* element of *D. melanogaster* (Daniels et al. 1990). This finding provided strong evidence for a recent horizontal transmission of a *D. willistoni*-derived *P* element into the gene pool of *D. melanogaster*.

In this review, we will elucidate in more detail the sequence relationships among *P* elements from a wide spectrum of taxa, their structural variation, and their evolutionary fate in different lineages. At the center of our considerations stands the *D. obscura* species group, which for many decades has served as a model system for phylogenetic studies (Lakovaara et al. 1976; Cabrera et al. 1983; Cariou et al. 1988; Goddard et al. 1990; González et al. 1990; Brehm and Krimbas 1993; Barrio et al. 1994; Barrio and Ayala 1997; Watabe et al. 1997; Ramos-Onsins et al. 1998; O'Grady 1999). The *obscura* group comprises 38 species, which can be assigned to five major subgroups (Barrio et al. 1994): *obscura*, *subobscura* (both Palearctic), *pseudoobscura* (Nearctic), *affinis* (Nearctic, with the exception of the Palearctic *D. helvetica*), and *microlabis* (Africa). Although the phylogenetic relationships of some species within the subgroups are not yet completely resolved, the *obscura* group provides an excellent framework for the analysis of *P* element evolution.

P element subfamilies and *P* element derivatives

P elements closely resembling that of *D. melanogaster* were not only found in *D. willistoni*, but also in several other species of the *willistoni* and *saltans* groups (Clark and Kidwell 1997). These *P* elements, referred to as "canonical" *P* elements, form a compact subfamily with a maximum sequence difference of 10%. Beside this subfamily, more diverged copies were detected in a wide range of taxa. The first of these "non-canonical" *P* elements (*PS18*) was isolated from the genome of *Scaptomyza pallida* (Simonelig and Anxolabéhère 1991). *PS18* represents a full-sized copy with the same general structure as the canonical *P* element but 24% divergence at the nucleotide level. When transferred into *D. melanogaster*, *PS18* proved able to integrate into the genome and induce excision of a defective *D. melanogaster P* element. Another full-sized *P* element related to *PS18* was later found in *D. bifasciata*, a species of the *obscura* group (Hagemann et al. 1992). Further screening of this species group revealed the existence of two other types of full-sized *P* elements, which are clearly distinct from the previously described *P* sequences (Hagemann et al. 1994, 1996a). Thus, besides the canonical *P* element, there are at least three well-characterized subfamilies designated as M-, O-, and T-type (Hagemann et al. 1996b). The sequence difference among the three subfamilies at the DNA level is about 30%, but the basic structure of four exons and terminal inverted repeats is the same (Fig. 1). The M-type subfamily is closest to the canonical *P* elements, whereas the O-type represents the most aberrant form, with interrupted terminal inverted repeats.

In a large scale polymerase chain reaction screening for an internal 449 bp section of exon 2, Clark and Kidwell (1997) analyzed 239 partial *P* element sequences from 40 species of the four principal species groups (*willistoni*, *saltans*, *melanogaster*, and *obscura*) of the subgenus *Sophophora*. A phylogenetic analysis reveals several differentiated clades. Some of these clades clearly correspond to the four subfamilies (canonical, M, O, T) mentioned above (Haring et al. 2000), others probably represent new subfamilies, but cannot yet be classified as such since only a small internal section has been analyzed and, especially, information about the type-characteristic termini is lacking. Nevertheless, these results indicate that a wide variety of distinct *P* element sequences are present in a limited sample of taxa.

Full-sized transposons, however, are not the only class of *P*-related sequences. Truncated *P* element derivatives have been detected in several lineages. In the *obscura* group, the genomes of the three species *D. guanche*, *D. subobscura*, and *D. madeirensis* harbor tandem repetitive arrays of *P*-related sequences (Fig. 1), which lack the terminal inverted repeats as well as the last exon (Paricio et al. 1991, 1996; Miller et al. 1992). Sequence comparisons identified these *P* derivatives as defective members of the T-type subfamily. Another truncated type, the *P-tsa* element, has a similar structure (lack of termini and exon 3) and has originated independently in the *montium* subgroup of the *D. melanogaster* group (Nouaud and Anxolabéhère 1997). Outside the genus *Drosophila*, apparently immobilized *P* homologs without terminal inverted repeats were discovered in the blowfly *Lucilia cuprina* (Perkins and Howells 1992) and in the house fly *Musca domestica* (Lee et al. 1999). In both sequences exon 2 is interrupted by two additional small introns. The reading frames are not intact and, thus, do not code for a functional protein. Exon 3 is present only in the sequence of *L. cuprina* (*Lu-P1*), but not in that of *M. domestica*. These immobile *P* homologs of *L. cuprina* and *M. domestica* may be considered as terminally truncated derivatives of once transpositionally active *P* elements, like those found in the *obscura* and *montium* species. Alternatively, one could hypothesize that they re-

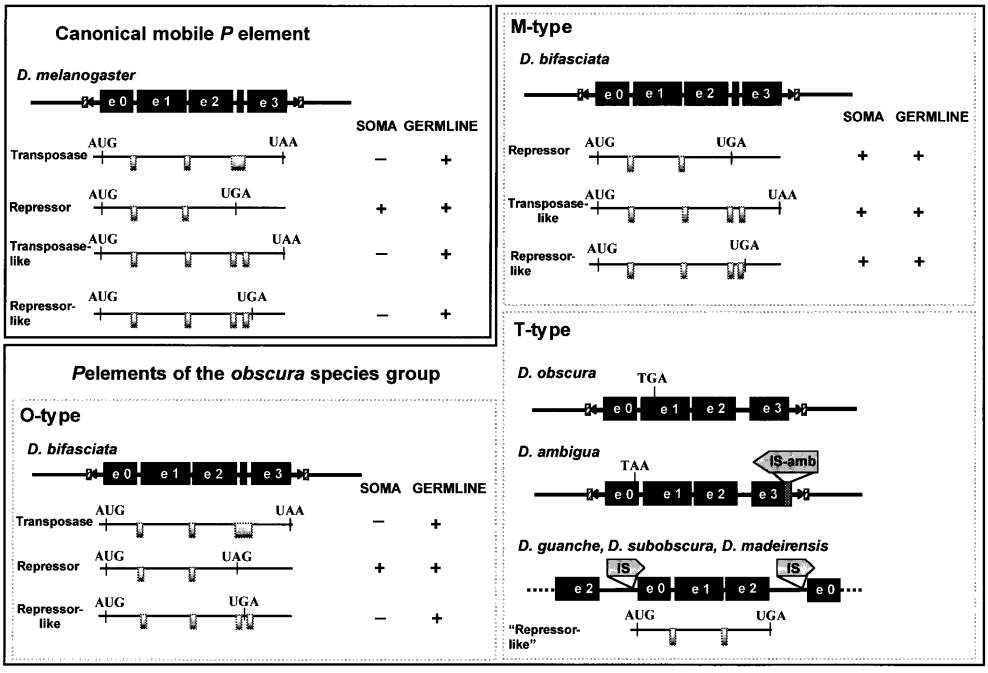

Fig. 1 Structure and transcription patterns of various *P* element types. The canonical *P* element of *Drosophila melanogaster* and the O- and M-type *P* elements of *D. bifasciata* share the same basic structure with four exons and terminal inverted repeats. A small additional exon is found in the intervening sequence between exon 2 and 3. Differential splicing of the transcripts results in four different mRNAs. However, germline-specific transposase mRNA is produced only by the transpositionally active canonical *P* elements and O-type *P* elements. The T-type subfamily comprises full-sized elements with defective reading frames (*D. obscura*, *D. ambigua*) and terminally truncated *P* element derivatives (*D. guanche*, *D. subobscura*, *D. madeirensis*). The latter are transcribed and give rise to a "repressor-like" protein with a novel function. *IS* and *IS-amb* are mobile insertion sequences of the *SGM* family

present descendants of an ancestral genomic progenitor sequence that later evolved into mobile *P* elements by acquisition of the terminal structures essential for transposition.

Horizontal transmission of *P* elements

P elements increase their copy number within the host genome through transposition and gap repair while sexual reproduction guarantees further propagation at the population level (reviewed in Engels 1989). Accordingly, the spread of *P* elements should be confined by reproductive barriers if transmission is restricted to a vertical mode. In contrast to this assumption the distribution of *P* elements among their host species is patchy and their sequence phylogeny is not congruent with that of their hosts. Both observations suggest that *P* elements have occasionally jumped over species borders. The horizontal transfer of the canonical *P* element from *D. willistoni* to *D. melanogaster* (Daniels et al. 1990) was the first convincing example. Additional horizontal *P* element transfers have to be postulated for the *obscura* species group. Among the three *P* element subfamilies detected so far, only the T-type shows a distribution pattern reflecting the phylogenetic relationships of the host species (Fig. 2). T-type *P* elements occur in five species of the *obscura* subgroup and, in truncated form, in three species of the *subobscura* subgroup (Miller et al. 1997; Haring et al. 1998a). Hence, T-type elements were likely present in the ancestor of these two subgroups. In the further radiation T-type *P* elements were transmitted vertically, but were eliminated from their host genomes in the *D. bifasciata*, *D. sinobscura*, and *D. alpina* lineages (Fig. 2) (Haring et al. 1998a).

The situation is different for the M- and O-type *P* elements, which are found sporadically in distantly related clades, suggesting that both types may have entered their host lineages horizontally. To trace their origins, the genomes of various drosophilids were screened, including the related genera *Scaptomyza* and *Lordiphosa*. *P* elements of the M-type subfamily were detected within the genus *Drosophila* (*montium* subgroup of the *melanogaster* group), but also in several species of *Scaptomyza* and *Lordiphosa* (Simonelig and Anxolabéhère 1991; Hagemann et al. 1994, 1998a; Haring et al. 2000). Sequence divergences among these elements range from 3.5% to 8.9%, indicating that horizontal transmission must have occurred in the more remote past. Although it is not possible to reconstruct the interspecific transmission routes in detail, at least three horizontal transfer events have to be invoked to explain the present distribution pattern (Haring et al. 2000). Even more striking are the sequence similarities (divergences <0.6%) among O-type *P* elements from three different genera (Hagemann et al. 1996b; Haring et al. 2000). This type originated in the ancestor of the American *willistoni* and *saltans* groups of *Drosophila* and was inherited vertically in both lineages.

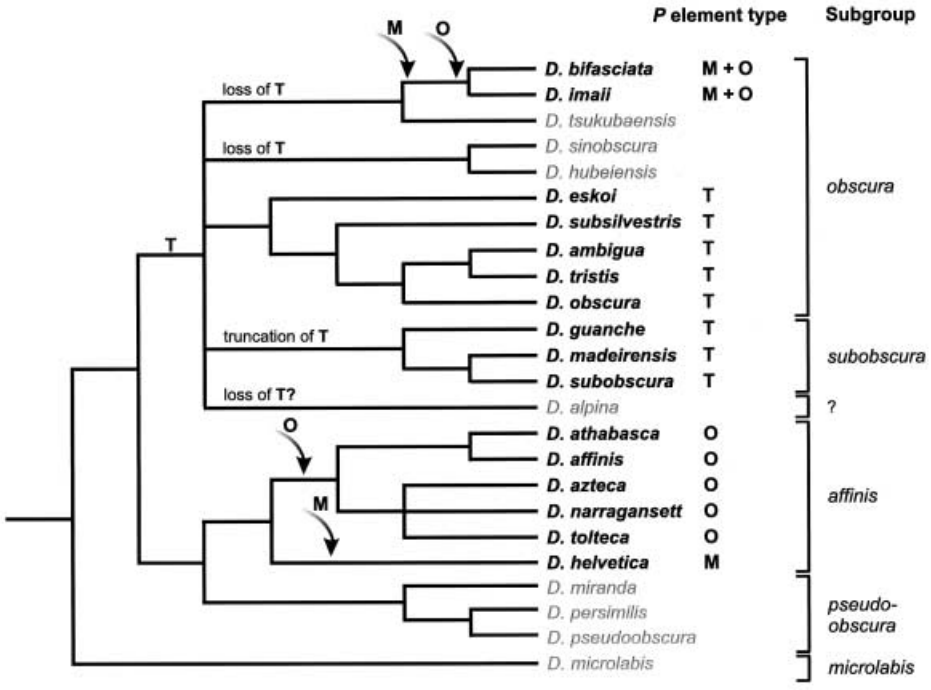

Fig. 2 Distribution of *P* element types in species of the *obscura* group of the genus *Drosophila*. The genomes of the 24 species were screened for the presence of the three types (O, M, T) using type-specific polymerase chain reaction primers. Invasions by O- and M-type *P* elements are indicated at the respective branches. Species relationships are depicted according to Haring et al. (1998a)

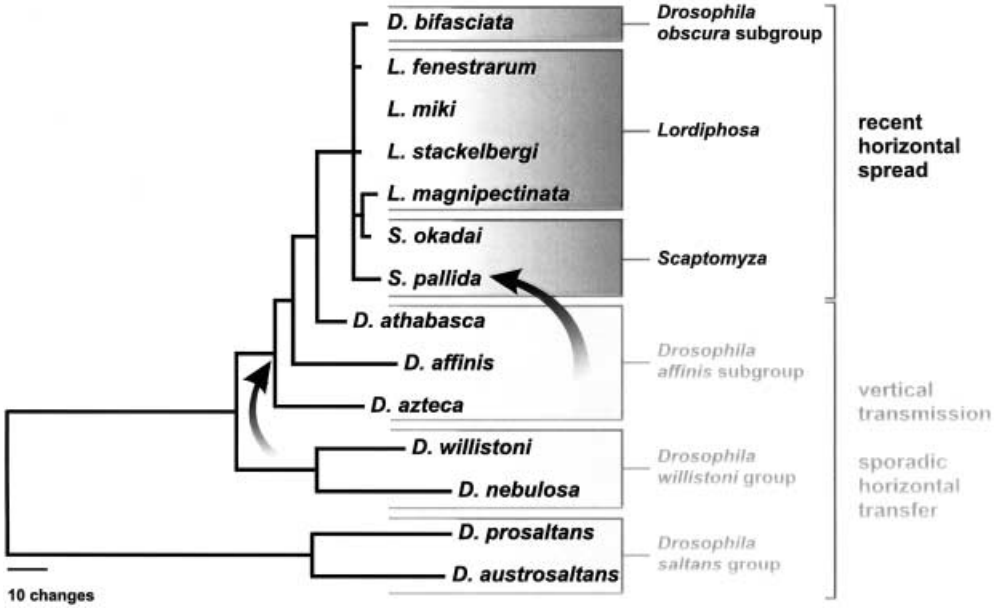

Fig. 3 Sequence relationships among O-type *P* elements are not in accordance with the phylogeny of their host species. In the lower part of the dendrogram vertical transmission dominates although two horizontal transfers (indicated by *arrows*) have to be postulated: (1) from the *willistoni* group to the ancestor of the *affinis* subgroup, and (2) from the *affinis* subgroup to the genus *Scaptomyza*. From there O-type *P* elements invaded the lineage of *D. bifasciata* and the genus *Lordiphosa* horizontally in a recent wave of interspecific transfers. Within these newly infected lineages *P* elements are again transmitted vertically

From there the O-type was first transferred to the lineage of the North American *affinis* subgroup (Fig. 3). After a longer period of vertical inheritance within this subgroup, a new wave of horizontal transmission led to the infection of several taxa of the genera *Drosophila*, *Scaptomyza* and *Lordiphosa* whereby the transfer over the Atlantic to Europe was probably mediated by the cosmopolitan species *S. pallida* (Haring et al. 2000).

The results summarized above provide strong evidence for at least eight horizontal transmission events between drosophilid species. Although alternative explanations (e.g., strong conservative selection, variation in evolutionary rates, ancestral polymorphism, and stochastic loss) should be considered first before horizontal transfer is postulated (Capy et al. 1997), these cases appear unequivocal because the transfers have occurred quite recently between distantly related taxa. For example, *D. melanogaster*, a species thought to have evolved in West Africa, did not come into contact with the *P* element donor *D. willistoni* before 1800, when *D. melanogaster* was introduced to America by humans (David and Capy 1988; Engels 1992). Thus, the transfer event must have occurred within the last 200 years. The alternative explanation, presence of the canonical *P* element in the

common ancestor of the two taxa, appears simply unbelievable. Even under the strongest conservative selection regime it is highly unlikely that only a single substitution should have occurred since the split of the *D. willistoni* and *D. melanogaster* lineages, estimated at 36 million years ago (Russo et al. 1995). The same argument applies for the other cases where the divergence of the host species or genera has to be dated much earlier.

If horizontal transfer is possible between distantly related hosts, it seems even more likely to occur between closer relatives. This, however, is more difficult to prove because diversification of *P* element sequences and cladogenesis of the host species take place in the same time frame. Silva and Kidwell (2000) have analyzed distribution and sequence relationships of canonical *P* elements in the *willistoni* and *saltans* groups. Comparing *P* element evolution with that of the *Adh* gene, they estimate that a minimum of 11 horizontal transfer events are necessary to explain the present distribution pattern.

Taking these results together, it can be concluded that horizontal transmission of *P* elements might be more common than anticipated. An interesting fact is that particular transfer routes are obviously preferred. *D. bifasciata* as well as species of the genus *Lordiphosa* have experienced two successive waves of horizontal invasions: first, by the M-type and, more recently, by the O-type subfamily. In both cases, the transmission probably involved *S. pallida* as an intermediate host (Hagemann et al. 1996b; Haring et al. 2000). Moreover, the interspecific exchange routes of transposons between drosophilids are not unidirectional. Jordan et al. (1999) proved that *D. willistoni* must have received the LTR retrotransposon *copia* from *D. melanogaster*. Accordingly, representatives of two different classes of mobile elements have crossed this species barrier within the rather short period of 200 years. Prerequisites for horizontal transmission are overlap of the geographic and ecological range, genomic host factors for successful integration and propagation, and an appropriate vector. One candidate that might have served as a vector for *P* element transfer is the semiparasitic mite *Proctolaelaps regalis*. Houck et al. (1991) showed that these mites are able to acquire *P* element sequences when feeding on *Drosophila* eggs under laboratory conditions. In this case, mechanical transfer of *P* elements between eggs using the digestive tract of the mite as a shuttle system might be considered as the most likely transmission mode (Kidwell 1993), as this means of infection mimics the widely applied method of germline transformation by microinjection (Spradling and Rubin 1982). Although this mode of transmission appears plausible, it has not yet been proved experimentally. Another promising vector is the α-proteobacterium *Wolbachia*, a widespread intracellular parasite of arthropods (Werren et al. 1995; Jeyaprakash and Hoy 2000) that infects gonadal cells. While cytoplasmically inherited within a host species, there is also evidence for frequent horizontal transmission of *Wolbachia* among different species via parasitoid wasps (Vavre et al. 1999). The presence of a mobile insertion sequence in the genome of a *Wolbachia* strain has been detected by Masui et al. (1999), suggesting that this bacterium could serve as a shuttle for transposable elements. This hypothesis is currently being investigated in our laboratory (Miller, unpublished results). Viruses are also considered as a likely vector system (Miller and Miller 1982). Baculoviruses, a group of DNA viruses that infect the larvae of insects (mainly Lepidoptera), have been shown to incorporate host transposons spontaneously into their genome (Jehle et al. 1998). The role of viruses in *P* element transmission among drosophilids, however, still remains to be explored.

Vertical inactivation

In *D. melanogaster*, transpositional activation of the canonical *P* element occurs when full-sized elements are introduced into a naive gene pool devoid of repressing mechanisms. This is achieved either by dysgenic crosses or germline transformation and usually causes an initial burst of *P*-induced mutations ranging from insertions to chromosomal rearrangements (Engels 1989). Since *P* element transposition causes double-strand breaks, the successful propagation within a newly infected host genome depends on the efficiency of the DNA repair system of the host (Quesneville and Anxolabéhère 1997). In addition, the increase in copy number seems to be counterbalanced by the capacity of the host to recognize and to silence the new invader (Kimura and Kidwell 1994; Kidwell and Evgen év 1999). These control mechanisms seem to be highly efficient since, in spite of the fact that eukaryotic genomes are crowded with parasitic DNAs, most of these sequences are transcriptionally inactive.

There is increasing evidence that transposons become silenced via epigenetic mechanisms such as DNA methylation, heterochromatization and post-transcriptional cosuppression (for recent review see Matzke et al. 1999). Epigenetic silencing mechanisms were found throughout all living kingdoms and are considered now as an ancient evolutionary defense mechanism against invading genomic parasites (Bingham 1997; Yoder et al. 1997; McDonald 1998). In plants and mammals, methylation of parasitic DNAs seems to be the most abundant silencing mechanism acting on parasitic DNAs (Wolffe and Matzke 1999). Although *Drosophila*, like many other invertebrates, is devoid of DNA methylation, mobile DNAs are under the regulatory control of epigenetic mechanisms, i.e., homology-dependent *trans*-silencing and post-transcriptional cosuppression. As shown by Dorer and Henikoff (1994), euchromatic tandem copies of *P* element transgenes become silenced by a homology-dependent epigenetic mechanism that is similar to position-effect variegation (Dorer and Henikoff 1994, 1997). Additional cases of cosuppression of parasitic DNAs in *Drosophila* acting at the transcriptional and post-transcriptional level have been reported recently (Pal-Bhadra et al. 1997, 1999; Jensen et al. 1999).

Hence it is intriguing that the inactivation of *P* transposon mobility might be also regulated at the epigenetic level. In *D. melanogaster* populations the most effective regulatory mode of *P* element repression is the maternally transmitted *P* cytotype (Engels 1979). Ronsseray et al. (1996) have shown that a single full-sized *P* element located within the subtelomeric region 1A of the *X* chromosome is sufficient for repressing *P* mobility in *trans*. This *trans*-silencing effect depends on the presence of the host-encoded heterochromatin-specific protein factor HP1, suggesting that the manifestation of the stable *P* cytotype in natural populations of *D. melanogaster* is caused by chromatin modifications. Thereby this mechanism ensures transcriptional silencing of all *P* element sequences present within a stable *P* strain (Roche and Rio 1998; Ronsseray et al. 1998). Altogether *P* transposon mobility is restricted by various factors exerted by the element itself as well as by the host genome, i.e., expression of autoregulatory 66 kDa *P*-repressor proteins (Misra and Rio 1990; Gloor et al. 1993), accumulation of defective copies (Black et al. 1987; Gloor et al. 1991), post-transcriptional repression via antisense *P* mRNA expression (Simmons et al. 1996), and epigenetic silencing via chromatin modification (Roche and Rio 1998; Ronsseray et al. 1998).

In the course of their coexistence with their respective host genomes, transposons are subjected to short bursts of activity followed by long-term inactivation (Bingham 1997; McDonald 1998). Therefore, in the long run unexpressed transposons are doomed to genomic extinction. As a result of recombination and random genetic drift the copy number will decrease and finally the immobile transposons will be eliminated from the gene pool (Fig. 5). But even those copies transmitted over many generations will suffer degradation of their information content. Without selective constraints maintaining the functionality of the sequence, an immobilized transposon will degrade by accumulating mutations, a process that finally leads to "vertical inactivation" (Kaplan et al. 1985; Lohe et al. 1995; Miller et al. 1996).

In the *obscura* group, examples of vertical inactivation can be observed among the M-type and T-type *P* elements (Fig. 2). None of the T-type elements analyzed so far have retained the coding capacity for functional transposase. Their genomic copy number is low and even in full-sized copies (*D. obscura*) the reading frames are destroyed by point mutations. Some species possess only internally deleted T-type *P* elements that lack large sections of the coding region (Hagemann et al. 1996b; Haring et al. 1998a). In the full-sized T-type *P* element of *D. ambigua*, exon 3 is interrupted by the short mobile insertion sequence *IS-amb* (Hagemann et al. 1998b). This insertion probably generated a defective *P* element that was no longer able to produce transposase but instead coded for a truncated protein that acted as a repressor of transposition for the remaining *P* element copies. After the T-type elements had lost their mobility, even this coding function became obsolete and the rest of the sequence began to degenerate. In the three species of the *subobscura* subgroup (Fig. 2), transpositional inactivation was probably brought about in a similar way by the destruction of exon 3, but in this case the coding function of the anterior exons (0–2) was maintained by new selective constraints (Miller et al. 1997) as will be described below.

The M-type *P* elements in the *obscura* group are confined to the closely related species pair *D. bifasciata*/*D. imaii* and to *D. helvetica*, the only representative of the *affinis* subgroup in the Palearctic. Both lineages have acquired their M-type *P* elements through horizontal transmission (Hagemann et al. 1992; Haring et al. 1998a). In *D. helvetica*, *P* elements are located at a single euchromatic site (Haring 1997), suggesting that they are transpositionally inactive. Nevertheless, the reading frames are intact and transposase mRNA is produced in germline cells (Haring et al. 1998b). Apparently these *P* elements have entered the gene pool of *D. helvetica* rather recently but, peculiarly, were not able to spread further in the genome. Whether the single site contains only one copy that was "dead on arrival" (the terminal inverted repeats have not been sequenced) or transposition is inhibited by some other mechanism has not yet been investigated. In contrast to *D. helvetica*, the lineage of *D. bifasciata* and *D. imaii* has experienced two successive waves of *P* element invasion, first by the M-type and later by the O-type in the course of the recent global expansion through horizontal transfer (Fig. 3). In *D. bifasciata* the O-type *P* elements appear to be still transpositionally active, as indicated by variation of the chromosomal locations among geographic strains, whereas the M-type elements are restricted to two euchromatic sites and thus are considered to be immobile (Haring et al. 1995). These differences in mobility between the two coexisting *P* element types can be attributed to differential splicing patterns of their transcripts controlled by the host genome (Haring et al. 1998b). Although both types are transcribed, germline-specific transposase mRNA is produced only from O-type transcripts but not from M-type. In contrast, repressor mRNA is made from both types. The situation is similar in *S. pallida*, where also apparently immobile M-type elements coexist with mobile O-type elements (Haring et al. 1998b). Therefore, although the M-type elements still carry the genetic information necessary to produce transposase and, in the case of the *S. pallida* M-type elements, are able to do so when transferred into the genomic background of *D. melanogaster* (Simonelig and Anxolabéhère 1991), transpositional activity is suppressed because transposase formation is inhibited at the level of mRNA splicing. These results suggest that the splicing machinery of the host is obviously able to distinguish between transcripts of different *P* element types, thus regulating their transposase production and genomic mobility.

Although immobilized transposons are, in the long run, exposed to degradation, such "sleeping" transposon variants may incidentally escape from silencing. The upper temporal limit for successful reactivation of a silenced gene has been estimated as 6 million years

(Marshall et al. 1994). At least four distinctive exit strategies to avoid silencing-induced sequence erosion are known: (1) the resurrection of silenced transposons by means of interelement selection and ectopic recombination between distinct subfamily members (Jordan and McDonald 1998), (2) the reactivation of silenced genomic parasites by stochastic loss of host-encoded suppressor alleles (Pelisson et al. 1994; Prud'homme et al. 1995), (3) the escape via horizontal transfer into the naive genome of an unprotected host species (Flavell 1999), and (4) molecular domestication (Miller et al. 1992; see below). Recombination between different elements has been observed recently, first in retrotransposons (Jordan and McDonald 1998; Saxton and Martin 1998), retroviruses such as HIV (Gao et al. 1998; Laukkanen et al. 2000), and also in DNA transposons (Miller et al. 1999; Peronnet et al. 2000). Sequence analyses of genomic parasites derived from yeast, *Drosophila*, mouse, and humans have shown that new active copies were generated by ectopic recombination between different variants present in the genome. In the case of retroelements the new active transposon variants most likely emerged from template switching of the reverse transcriptase between two or more parental subvariants giving rise to new hybrid cDNA molecules. Hybrid elements of DNA transposons were detected recently. In *D. melanogaster* the resurrection of an active canonical *P* element through recombination of defective copies has been described by Peronnet et al. (2000). In the *obscura* group, sequence comparisons indicate a hybridogenic origin of the T-type *P* elements (Miller et al. 1999). Thus the formation of novel, activated hybrid transposons generated by ectopic recombination between elements from different subfamilies seems to be an effective mechanism to escape recognition and silencing by the host genome.

Molecular domestication

The transition from a parasitic sequence to a beneficial host gene has been described as molecular domestication (Miller et al. 1992). The first example of a domesticated DNA transposon is the stationary *P* element-related neogene cluster of the *obscura* group species *D. guanche*, *D. subobscura*, and *D. madeirensis* (Paricio et al. 1991; Miller et al. 1992). In these species terminally truncated *P* element derivatives related to the T-type subfamily are tandemly repeated at a single euchromatic site (Fig. 1). They have lost their terminal inverted repeats and the transposase-specific exon 3, but retained the coding section comprising exons 0–2, which is translated into a "*P*-repressor-like" protein (Fig. 4). The three domains known to be of functional significance for canonical *P*-repressor activity (Rio 1990; Andrews and Gloor 1995; Miller et al. 1995; Lee et al. 1996, 1998), i.e., the N-terminal zinc finger motif, the coiled-coil domain and all three leucine zippers are conserved. A detailed model describing the formation of the *P*-neogene cluster of *D. guanche* and related species step by step has been proposed by Miller et al. (1997). The homologous genomic position of the *P*-neogene cluster in *D. guanche*, *D. madeirensis* and *D. subobscura* indicates that the loss of mobility took place in the common ancestor at least 3 million years ago. It can be assumed that the truncated copies originally functioned as a stable source of repressor protein that inhibited further transpositional activity of intact *P* elements. However, the coding regions of these truncated elements are still conserved although mobile *P* elements have by now completely disappeared from the genome. This raises the question whether they may have acquired a novel function and consequently are maintained by new selective constraints. This view is supported by the fact that transcription of the cluster units is now under the control of a novel *cis*-regulatory section generated by insertions of mobile sequences not related to *P* elements (Miller et al. 1995, 1997; Hagemann et al. 1998b).

P element derivatives with a similar molecular structure (Fig. 4) occur in *D. tsacasi* and several related species of the *montium* subgroup (*melanogaster* group) (Nouaud and Anxolabéhère 1997). As the stationary *P*-neogene of *D. guanche* and its relatives they lack the terminal and subterminal inverted repeats and exon 3 but have conserved the coding capacity for a *P*-repressor-like polypeptide over evolutionary time (Fig. 1). In contrast to the clustered organization of the *P* derivatives in *D. guanche*, the stationary *P* derivatives of the *montium* subgroup exist as highly conserved single-copy genes. Neither their chromosomal location nor the flanking sequences are homologous to those of the clustered *P* derivatives in *D. guanche* and its relatives. This indicates that these truncated *P* derivatives have originated independently in the *montium* lineage. The *P* domestication event in the *montium* subgroup predates that in the *obscura* group. Since the *montium* *P*-neogenes are highly conserved at orthologous genomic positions in all spe-

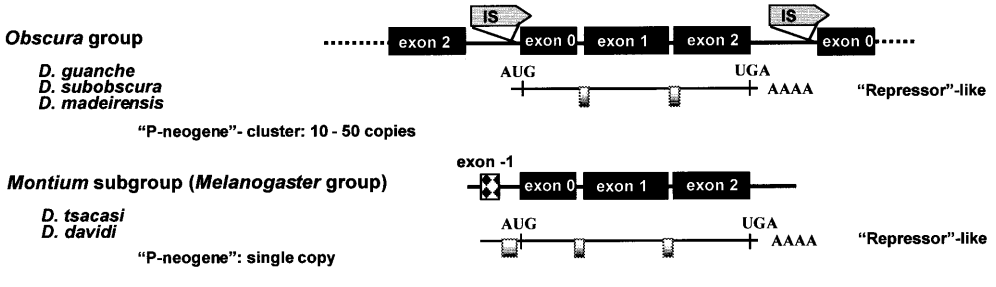

Fig. 4 Immobilized *P* element derivatives in the genus *Drosophila*. Terminally truncated *P* derivatives originated independently in species of the *obscura* group and the *montium* subgroup. Both types of neogenes consist of exons 0–2, thus coding for repressor-like proteins, but differ in their 5′ sections. (*IS* insertion sequence)

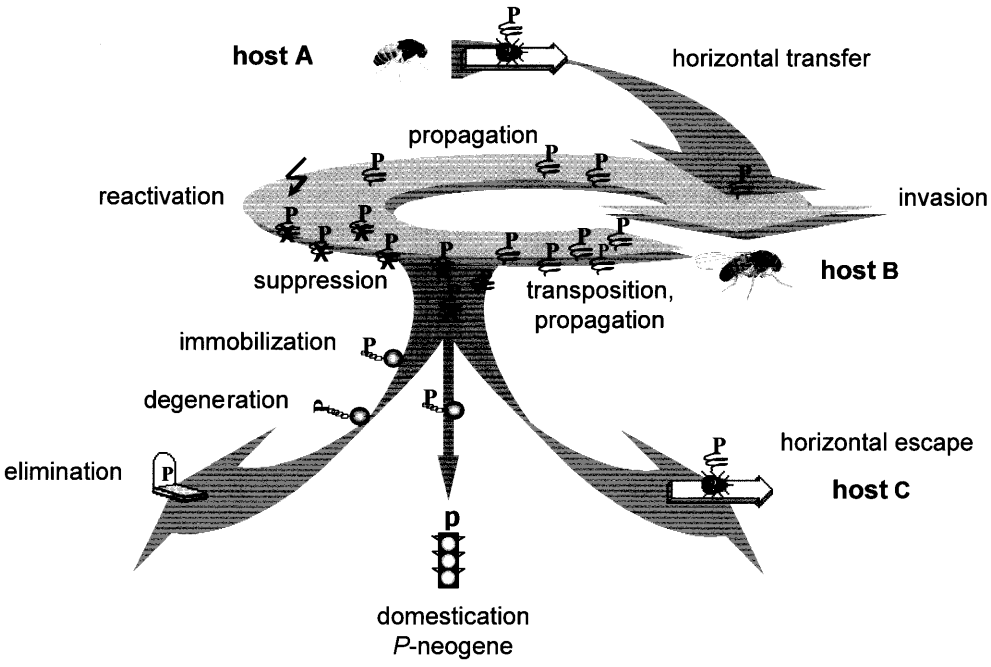

Fig. 5 Evolutionary life history of *P* transposons: this model starts with a *P* element infection via horizontal invasion of a naive gene pool mediated by still unknown vectors (here symbolized by a mite). After an initial transpositional burst that leads to rapid propagation in the genome as well as in the population, transpositional activity is suppressed by various mechanisms. Immobilized *P* elements are either reactivated to start a new period of genomic mobility or are subjected to molecular erosion by random mutations. Genomic extinction can be avoided by horizontal escape into an uninfected gene pool where a new cycle is initiated. A sidetrack from this cycle leads to molecular domestication and acquisition of a novel host-encoded and perhaps regulatory function (symbolized by the *traffic light*)

cies of the *montium* subgroup but are absent in other subgroups of the *melanogaster* group, it is conceivable that their loss of mobility took place in the common ancestor of the *montium* subgroup approximately 20 Mya (Nouaud et al. 1999).

Although both types of *P*-derived neogenes are transcribed in adult flies into polyadenylated RNAs encoding *P*-repressor-like proteins, the *cis*-regulatory sections driving their individual expression have different origins (Fig. 4). In the case of the *obscura P*-neogene, a novel promoter region evolved from an insertion sequence (*IS* in Figs. 1 and 4), a MITE-like transposon of the *SGM* transposon family related to *IS-amb* (Miller et al. 2000). Activation by this captured promoter might have resulted in a different expression pattern, initiating a novel function of the protein (Miller et al. 1995, 1997, 2000; Hagemann et al. 1998b). In contrast, the 5′ regulatory section of the *montium P*-neogene might have evolved from intergenic sequences flanking the *P* element derivative. Surprisingly, the *montium P*-neogene contains a new exon (exon –1) and a new intron (intron –1) upstream of the original *P* element insertion site (Fig. 4). This nontranslated exon –1 originates from flanking sequences at the genomic insertion site of a mobile *P* element, whereas the first half of the new intron consists of genomic flanking sequence and the second half is composed of a *P* element-derived sequence (Nouaud et al. 1999). Although the functional properties of these *P* element-derived neogenes in their respective hosts are still unknown, this system provides the first case for the multiple independent acquisition of transposon-derived coding sections during *Drosophila* evolution (Nouaud and Anxolabéhère 1997; Miller et al. 1999).

Evolutionary life history of *P* elements

The different stages in the evolutionary life history of *P* elements, exemplified by the different *P* element subfamilies in the *obscura* group and other drosophilids, are summarized in Fig. 5. After an initial transpositional burst with rapid propagation in the genome as well as in the gene pool, mobility comes to a halt by the successive accumulation of defective copies and host-directed epigenetic silencing. Subsequently, the intact elements coding for functional transposase are lost from the genome and thus the immobilized copies are no longer under selective constraints. Exposed to random mutations, they undergo sequence erosion and are finally eliminated from the host genome unless reactivation within the original host genome is brought about either by interelement recombination or suspension of the repressing regime. Another possible escape route is the invasion of an uninfected gene pool via horizontal transmission and the beginning of a new cycle in a permissive genomic environment. Finally, survival as an intact coding sequence can be achieved by molecular domestication, the stable integration into the genome and acquisition of a novel function that benefits the host organism. However, molecular domestication differs from the other modes of preventing genomic extinction. Although, the sequences remain at least partially identifiable, they have lost the capacity to be members of a mobile element family. Accordingly, becoming a neogene has to be considered as a sidetrack rather than an integral part of the transposon life cycle. Interestingly, a growing body of evidence is just accumulating in support of our view that recruitment of transposable element derivatives is more than a sporadic episode in genome evolution (reviewed in Miller et al. 1999; International Human Genome Sequencing Consortium 2001).

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