Plant transposons: contributors to evolution

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Abstract

A spectrum of different hypotheses has been presented by various authors, from plant transposable elements as major agents in evolution to the very opposite, transposons as mainly selfish DNA constituting a genetic burden for the organisms. The following review will focus on: (1) a short survey of the two main different assessments of transposable elements (TEs) concerning the origin of species (selfish vs useful DNA); (2) the significance of the hierarchy of gene functions and redundancies for TE activities (selfish in non-redundant parts of the genome, but as a source of variability in the rest); (3) the relevance of the results of TE research in Zea mays and Antirrhinum majus for species formation in the wild (contrast between artificial and natural selection); (4) three areas of research where a synthesis between the two different evaluations of TEs seems possible: regressive evolution, the origin of ecotypes and the origin of cultivated plants; and (5) some possible prospects regarding TE-induced species formation in the angiosperms in general, i.e., the basic difference between systematic and genetic species concepts and the conceivable origin of a large part of angiosperm morphospecies owing to loss of function and further mutations by TE activities.

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1. Introduction

"... the notion that all the still functioning genes in the genome ought to be indispensable for the well being of the host should be abandoned once and for all (Ohno, 1985, p. 164)."

The discovery of transposable elements (TEs) by McClintock (1947) has been connected with several ideas about possible important functions in ontogeny and later also in the origin of species and higher systematic categories (McClintock, 1947, 1950, 1953, 1978, 1984, 1987). The main reason for this was the extraordinary potential of TEs to generate mutations of all kinds: deletions, insertions, frameshifts, inversions, duplications, translocations, larger genome rearrangements and the production of intron-like sequences (for reviews of the evidence, see, for example, Nevers et al., 1986; Döring et al., 1990; Walbot, 1992; Grappin et al., 1996; Saedler and Gierl, 1996; Kunze et al., 1997). Changes in the spatial and temporal gene expression as well as its strength belong to the repertoire of TE effects (see, for example, Coen et al., 1986, 1989; Schwarz-Sommer, 1987; Schwarz-Sommer et al., 1987; Hake et al., 1989; Hake, 1992; Bradley et al., 1993; Lönning and Saedler, 1994). Moreover, most of these mutations could be induced with such a high rate that hardly any other naturally occurring mutagenic agent has a chance to compete with TEs (for some numerical calculations, see Robertson, 1978; Nordborg and Walbot, 1995).

Assuming with the neo-Darwinian school that mutations are the raw material for the origin of all life forms on Earth (reviewed in detail, for example, by Lönning, 1993), it was only consistent that leading textbook authors such as Lewin (1994) and Alberts et al. (1994) have promoted the idea that TEs may provide a major source of mutations and are important for evolution. However, this notion was called into question some years ago when several authors presented their hypothesis about TEs as mainly selfish or even 'parasitic' DNA constituting a distinct genetic burden for the organisms...
harbouring them (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). Some of the arguments for and against the different hypotheses will be presented in the following paragraphs (for a more detailed analysis the reader is referred to the review by Kunze et al., 1997). As it is still possible for even the most successful transposon researchers to have widely diverging views on the questions of plant TEs and evolution, we have tried to present the main data and ideas as objectively as possible, indicate the open questions for further research and point out where a synthesis seems possible. In doing so, we invite the reader to decide which of the current hypotheses appears to be the best so far to explain the presence of TEs in plants.

2. Transposable elements as selfish DNA

Until Doolittle and Sapienza (1980) and Orgel and Crick (1980) presented their hypothesis that TEs might be selfish DNA, it was commonly assumed ‘that these sequences are an integral part of the genome and that their evolutionary origin was the result of Darwinian selection acting on the host organism phenotype’ (Hickey, 1993, p. 142). In contrast, the new hypothesis postulated that pieces of DNA without phenotypic expression ‘appeared’ and propagated in the genome. Such elements existed essentially for their own sake: no cellular functions were thought to be necessary as long as maintenance and mobility mechanisms existed. Among the original arguments presented for the selfish DNA hypothesis and against selection were: (1) TEs confer no immediate phenotypic benefit and thus no immediate selective advantage for the organisms harbouring them; (2a) evolution is not anticipatory, hence no structures are formed because they might later prove useful; (2b) a species’ adaptability due to TEs seems to be far too remote to explain the origin and maintenance of these sequences; and (3) it appears unlikely that the C-value paradox which can be due to an enormous difference of TE numbers in even closely related species could be explained by selection on the organism’s phenotype.

Orgel and Crick (1980) labelled such DNA ‘parasitic’ and even called it ‘the ultimate parasite’ because selfish DNA seems to be at least a slight burden for the respective organisms, and Doolittle and Sapienza (1980) emphasized that sometimes it is distinctly destructive. But why, then, do such sequences replicate and spread? The point is that not only do they exist in an environment where DNA replication is a necessary part of each cell division but, as later detected, many TEs also code for their own transposases necessary for autonomous movements. Hickey (1982) calculated that host fitness may be reduced to 50% by selfish DNA sequences, and yet they may spread through a population.

Following the papers just mentioned, Golding et al. (1986), Hartl (1988), Maynard Smith (1989), Charlesworth and Langley (1989), Preston and Engels (1989), Starlinger (1993), Maynard Smith and Szathmáry (1995) and Engels (1996) are among those authors finding further evidence backing up the selfish DNA hypothesis. Starlinger (1993), for example, assumes that the role of plant TEs as selfish DNA is ‘probable’, but as a major agent in evolution is ‘unlikely’.

3. Transposable elements as major agents in evolution

In contrast, numerous authors view the effects of TEs for the origin of species more in line with the ideas of McClintock mentioned in the Introduction (e.g., Schwarz-Sommer et al., 1985; Nevers et al., 1986; Coen and Carpenter, 1986; Coen et al., 1989; Flavell et al., 1994; White et al., 1994; Shapiro, 1995; McDonald, 1995; Wessler et al., 1995). The main reason for this assumption is the above-mentioned vast potential of sequence variation and functional alterations that TEs are capable of inducing (for further reasons and details, see Kunze et al., 1997). Because of these nearly unrivalled mutagenic effects, even most researchers focusing on the selfish DNA hypothesis think that it is conceivable that TEs might at least occasionally be involved in structure and/or species formation (e.g., Hickey, 1993). Moreover, Saedler (1991) has addressed the question of whether plant DNA transposons may have more capabilities to produce sequence variations due to exceptionally high reversion rates and footprint production in their hosts than the TEs found in animals. McDonald (1995) deduces from the current evidence that ‘the once popular notion that TEs are merely junk DNA and of no evolutionary consequence is no longer tenable’. However, there are some areas of research where a synthesis between the opposing views appears to be possible (see discussion below).

4. Transposons and the hierarchy of gene redundancies

As far as the authors are aware, this key point of the hierarchy of gene redundancies for an adequate assessment of the question posed in the title of the paper has not previously been brought into the discussion on TEs and evolution. Blixt (1972) distinguished (with reference to Ohno, 1970) between the ultraconservative part of the genome with genes for basic cell functions and differentiation mechanisms, where most of the genetic variation represents ‘forbidden mutations’ (mutants not viable at any developmental stage), a conservative part of the genome (lethals, sterile individuals, severe chlorophyll deficiencies, etc.), and the variable or redundant part (interesting, for example, for the plant breeder) ‘and in all probability this constitutes a minor part’ of the genes.
Concerning the effects of TEs this means that insertion and footprint formation in, for instance, genes coding for histones, actin and many tRNAs (for a series of further examples, see Lönnig, 1991; Behe, 1996) will almost always be destructive (selfish DNA), whilst the vast potential of sequence variation and functional alterations that TEs are capable of inducing might have some realistic chances as a source for variability in the more redundant parts of the genome. For the question posed in the title, the possible influence of TEs on the variants of the anthocyanin system, plant height, flowering time, shifts from perennial to biennial to annual life cycles, etc., in natural plant species could be rewarding objects of further research (ecotype formation and regressive evolution). Modification and especially inactivation of redundant gene functions could also, perhaps, be the essential contribution of TEs in cultivated plants (see below). These points would confuse the promising part of transposon activities essentially to what might be called microevolutionary events. Conversely, for any ‘forbidden mutations’, i.e., all those mutations with immediate lethal effects (or any disastrous consequences at least before sexual maturity), all transposon activities necessarily constitute purely ‘parasitic events’.

5. Zea mays, Antirrhinum and species formation in the wild

Most TE studies have been carried out with cultivated plants such as maize and commercial varieties of the snapdragon Antirrhinum majus L. In the discussions these studies have often also been connected with questions on the role that TEs might play in plant evolution. Although a wealth of scientific evidence for the consequences of TE activities has been described in these species, a simple and general extrapolation from the results obtained to questions regarding the origin of wild plant species is not as easy as perhaps expected: TE-induced genetic variation tolerated or even desired by humans and propagated under their guardianship is not necessarily identical to that required for species formation in the wild. Maize is fully dependent on human care and conservation, as are probably also all commercial varieties of Antirrhinum. Without the interset of humans, Z. mays ssp. mays (our common corn) would rapidly become extinct, as would most cultivated Antirrhinum lines. For example, hardly any of the splendid colour varieties of our cultivated A. majus lines have been detected in wild snapdragon populations. Rather, an extensive series of multiple alleles of the Rosea (Ros) gene is the most often found contribution to colour variation in the wild (Baur, 1933; Hackbarth et al., 1942; Stubbe, 1966; see also the rather monotonous, but correct colour descriptions for the 20 European Antirrhinum species plus several subspecies given by Sutton, 1988). However, a null allele of Sulphurea in A. latifolium as well as some other loss-of-function alleles in the cascade of colour genes appear to be involved in the natural colour variation of different species (Hackbarth et al., 1942; Günther and Rudolph, 1970). For these genes as well as the Ros locus it would be highly relevant to find out whether transposon activities have been involved (perhaps producing new alleles by footprint production or blocking gene functions by insertions) or whether ‘conventional’ mutations are the main cause.

The difference between the causes of species formation in regressive evolution in the wild (see below) and the results of the investigations in Z. mays and Antirrhinum may only be a question of which of the different redundant components of genomes have been changed or inactivated.

6. A possible synthesis: selfish DNA as a tool in microevolution

As indicated above, an assumed selfishness of TEs does not exclude their usefulness for certain kinds of genetic alterations in confined areas of systematic and applied biology. In the three fields of genetic research discussed in the following paragraphs selfish and parasitic TEs may prove to be a valuable tool for rapid genetic changes: gene inactivations in regressive evolution, the origin of ecotypes and the origin of cultivated plants. We focus our attention on different degrees of gene inactivations here as these changes are the most commonly found consequences of TE insertions in coding sequences and because the potential value of such inactivations has hardly been assessed in the literature so far. This concentration on loss-of-function mutations is not meant to exclude more subtle effects of TEs in these and other fields of research, although at present there is no concrete evidence for either of these possibilities in natural plant populations. (Equivalent functional allele variations resulting in altered proteins, for example, could be another but probably more difficult topic for our question posed in the headline: it will often not be easy to decide whether small DNA sequence variations should be ascribed to TE activities or to ‘normal’ mutations.)

6.1. Transposable elements and regressive evolution

By definition, regressive evolution has always been connected with the loss of some functions of anatomical and/or physiological structures (organ and pigment reduction in cave animals, loss of flying abilities in insects and birds on islands around the world, loss of
scales in fish species in closed lakes, loss of dispersion systems in island plants, etc.). In many of these cases, gene structures and functions normally essential for survival in the wild have become redundant under special environmental conditions. Some complex anatomical and physiological structures may even prove to be a heavy energetic burden in the new environments so that losses of function can have a positive selection value. As all authors agree that for coding sequences ‘most insertion events create a null phenotype’ (Walbot, 1992, p. 85) or in other words ‘the well-known mutations caused by transposable elements are in their majority gene inactivations’ (Starlinger, 1993, p. 253; see also Lönnig and Huijser, 1994), examples of regressive evolution might be outstanding candidates for further research on the question of to what extent TEs could be involved in genetic changes of natural populations.

For instance, recessive radial mutants of Antirrhinum and Linaria have long been the object of vigorous phylogenetic speculations (atavisms and de novo species formation in the wild; Figs. 1–3) (Gustafsson, 1979). However, zygomorphic plant species can give rise to distinct kinds of radial mutants differing strongly from one another in their morphology, so that several authors have rejected the interpretation of an atavism here (Penzig, 1922; von Goebel, 1928; Arber, 1970; Hartl, 1974; Endress, 1992). The species cannot have had differently looking ancestors at the same time. Besides, the radial flower form is often due to the five to six times repetition of a flower part already differentiated and synorganized for the zygomorphic flower (the middle part of the lower lip with lobe, gibba or spur and further structures) (Figs. 2 and 3) as well as total or nearly total suppression of the upper lip. Last not least, normal flower function is often strongly hampered by severe constriction of the entrance and other malformations and malfunctions (pollinators excluded, autogamy reduced, lower seed production and seed sterility, also, owing to nearly upright position, early fouling of all flower parts due to rain water in the flower under field conditions in Antirrhinum).

Nevertheless, such ‘prodigies’ may have played a part in regressive evolution (see below). One of the two genes whose recessive alleles give rise to such hemipeloric phenotypes in Antirrhinum has just been cloned by Luo et al. (1996), who named it Cycloidea. Note, however, that this gene is not identical to the one giving rise to the well-known cycloidea radialis mutant (Baur, 1910; Stubbe, 1966) displayed in Fig. 1. The investigations depicted in this figure demonstrate the extraordinary rapidity of TEs to induce these mutants as well as revertants probably due to new alleles (for details, see legend to Fig. 1; an example of the variation on the protein level of already molecularly investigated functioning alleles of other genes is shown in Fig. 4 for the Waxy locus of Z. mays).

Although there are no records of such cycloidea alleles as part of natural populations of Antirrhinum, a similar mutant (of the Cycloidea locus?) of the closely related genus Linaria appears to occur regularly and propagates vegetatively in natural populations on the islet Skärpskär of the Swedish archipelago of Roslagen, north-east of Stockholm and some other places: Linné’s (Linnaeus’) famous Peloria (Greek for ‘wonder’, ‘monster’ or ‘frightening sight’) (Linnaeus, 1749; de Vries, 1906; von Goebel, 1928; Gustafsson, 1979). Interestingly, de Vries (1906) obtained 119 descendents of peloric Linaria individuals, of which 13 plants were normal. If a transposon was involved this would be a reversion rate of 11%, a rate in agreement with values found in Antirrhinum (we found reversion rates between 2 and 23% for different alleles). It should be emphasized that such enormous morphological differences as shown in Figs. 1–3 can be due to the inactivation of just one gene. However, TE activities in non-redundant parts of the genomes involved in, or fully constituting, species (and especially higher category) differences would again form purely parasitic events. In all these cases non-redundancy may cover first and foremost the indispensable genetic functions for the existence of a species mentioned above, and additionally the capacities necessary to cope with special environments (‘relative functions’). For a review of the many open questions concerning species formation due to entirely new genes and reaction chains, see Kunze et al. (1997).

One general observation for regressive evolution is the extraordinary rapidity with which these processes often appear to have occurred independently at several places around the world (for examples, see Diamond, 1981; Lönnig, 1993, 1995). Thus, TEs could be ideal aspirants not only for the many gene inactivations involved in regressive evolution, but also for the rapid induction of the many independent mutations occurring in identical or similar gene loci of the same or different animal and plant species in places often far apart on the Earth. However, at present it is not known whether TEs are involved here.

6.2. Transposable elements and the origin of ecotypes

Ecotypes of the same species often display some adaptations to a special environment (different degrees of temperature and light/shadow tolerance, resistance to increasing salt concentrations and metal compounds in the soil, specializations for altitudes and lack or abundance of water, etc.). Just to glance briefly at the extensive topic of resistance: among the many different genetic causes of resistance there are numerous experimental examples, which involve gene inactivations (reviewed by Lönnig, 1993). The principle is very simple: specific toxic substances such as heavy metals or chemical compounds cannot be absorbed or incorporated any
Fig. 1. Strongly accelerated mutation rates at a specific locus due to TEs. (A) *Antirrhinum* flower of normal zygomorphic form, but with active Tam3 (Transposon *A. majus*) causing variegation of the red colour due to instability at the *Nivea* locus (for a review, see Coen et al., 1989). (B) The stable *cycloidea radialis* line from the Gatersleben collection of *Antirrhinum* mutants. The spontaneous mutation rate per gene per generation is generally given as $1 \cdot 10^{-5}$ to $1 \cdot 10^{-6}$. (C) Flower of normal F1 plant. Among 31,000 F1 plants there were five with new unstable *cycloidea* alleles (D), so that the mutation rate was strongly increased to $1.6 \cdot 10^{-4}$. (E,F) The descendants of the different new mutants displayed reversion rates between 2 and 23%, in the present case 4.8%. The reversion rate at the second locus was 0.34% (Carpenter and Coen, 1990; Luo et al., 1996). Such revertants are often due to alleles slightly differing from the wild-type sequence, so that also the phenotypes may be affected correspondingly. In strong contrast to these data, back-mutations as studied in bacteria are usually about 10 times less frequent (i.e. $10^{-8}$ to $10^{-9}$) than the "normal" forward mutations (Lewin, 1994).
more and the organism is thus resistant to them. Alternatively, the substance or compound becomes poisonous by chemical modifications in later steps of the metabolism. If such steps are lost, the compound is not metabolized to the poisonous state any more and again an organism becomes resistant to it. Hence, the question may be examined of whether resistances due to gene inactivations by TEs are relevant for natural populations.

Another question that should be thoroughly investigated is whether TE-induced sequence variations in Z. mays, such as those caused by the Cin4 transposon in the A1 gene (Schwarz-Sommer et al., 1987) or the gene duplications of the R-γ complex with putative TE footprints (Walker et al., 1995), can be extrapolated to useful mutation events in the wild, perhaps being involved in ecotype formation. As far as gene inactivations, formation of functional alleles and chromosome rearrangements are involved in such adaptations, TEs could again be good candidates for accelerating the mutation processes in the more redundant parts of the genomes. Thus, this appears to be another field of future research to decide the question of whether, and if so, to what extent, TEs are involved in genetic changes of natural populations.

6.3. Transposable elements and the origin of cultivated plants

In comparison to their wild ancestors, the new characters of diploid cultivated plants are in the large majority of cases recessive (von Sengbusch, 1980) (some maize features seem to be the best-known exception to this rule). Recessiveness can be equated with different degrees of loss of gene functions (Kacer and Burns, 1981; Fincham, 1983; Watson et al., 1987; Alberts et al., 1994). As most insertion events of TEs create a null allele or lead to partial inactivations of genes, the vast field of diploid cultivated plants could be another test case for the question of to what extent TEs are involved in useful genetic alterations (although these cases do not concern natural populations, but ‘only’ changes helpful to humans). Fortunately, there is already some molecular evidence for the involvement of TEs in gene inactivations here: Tam2 blocks gene function of the nivea locus in the culture variety ‘Snowman’ of A. majus (Hehl et al., 1987). The tubocolorata allele of the Pallida gene of a commercial line of A. majus is another case where a TE appears to be involved (Coen et al., 1986). Also, ‘the wrinkled seed character of the pea described by Mendel is caused by a transposon-like insertion in a
gene encoding starch-branching enzyme' (Bhattacharyya et al., 1990). Staying a bit longer with genetics' oldest experimental organism, further good candidates might be gene inactivations for features such as white flowers and green seeds, plant height and fasciation.

Concerning maize, Peterson (1993) has argued that genes must be reduced in activity to produce smaller tassels and less robust leaves in maize breeding so that future gene inactivations regarding such features by TEs accelerating the process could be helpful (large tassels and robust leaves are not necessary in most corn-breeding surroundings). Also, there appears to exist some evidence for a positive correlation between breeding success and TE activities for Uq. The definite answer for or against this conclusion, however, will be found by the isolation of genes which contribute to the performance of the populations (Peterson, 1993). Generally, it can be expected that a large number of suitable gene inactivations (partial and null alleles) might be due to TE insertions or TE-induced mutations including footprints. The calculation of the exact proportions of such gene inactivations in cultivated plants due to TE activities in relation to conventional mutations could be a major research project for the future.

6.4. Prospects of TE activities for the origin of angiosperm species

The angiosperms include approximately 13 400 genera with more than 250 000 species (in part calculated after Stace, 1989; Gunn et al., 1992; Stevenson, 1997). Interestingly, most species of thousands of angiosperm genera can produce hybrids with different degrees of fertility from fully sterile to perfectly fertile. To mention just two longstanding experimental plants of genetics: all 20 Eurasian and African Antirrhinum species yield fertile hybrids and the same is true of the seven Pisum species distinguished in botanical systematics according to morphological criteria. From a strictly genetic species concept both cases could be addressed as monospecific genera. Moreover, natural hybrid swarms are known in many plant genera. Kerner von Marilaun (1891) was able to work out a list of more than 1000 interspecific wild hybrids of European plant species, the majority of them angiosperms. Today, 'a complete list of natural hybrids and hybrid swarms in plants would run from Achillea to Zinnia' (Grant, 1981, p. 197: for a further discussion of the evidence see, for example, Scherer, 1993; Arnold, 1997). Different morphological species of an angiosperm genus are, from the genetic point of view, often nothing but special recombinants of one and the same species. This inference can also be applied to many plant genera (for a detailed discussion, see Lönnig, 1993). Typically, the differences between such 'species' consist of alleles segregating in the Mendelian fashion, including null alleles. It is in this kind of species formation, incorporating literally tens of thousands of plant species all over the world, where again TEs, with their extraordinary potential to rapidly produce null and other alleles, could have played a role in the redundant parts of the genomes. Although a large part of that kind of species formation will again be classified as regressive evolution, an investigation into the extent to which TE activities are involved in the variation of wild plant populations appears to be a worthwhile enterprise. On the genetic level TEs could be involved in microevolution, a part of which could mean 'transspecific' evolution on the systematic level (Lönnig, 1986).

Especially with regard to regressive evolution, ecotype formation and the origin of cultivated plants, Ohno's motto quoted at the beginning of our paper can be extended to 'the notion that all the still functioning genes in the genome ought to be indispensable under all environmental circumstances for the well-being of the host should be abandoned once and for all'. In conclusion, research is necessary to show definitively to what extent TEs have been involved in the origin of cultivated plants and whether TEs have played a role in natural populations or not.

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