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Genetic and Epigenetic Changes Involving (Retro)transposons in Animal Hybrids and Polyploids

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Hybrid dysgenesis · Retrotransposon · RNA-mediated silencing · Transposable element

Abstract
Transposable elements (TEs) are discrete genetic units that have the ability to change their location within chromosomal DNA, and constitute a major and rapidly evolving component of eukaryotic genomes. They can be subdivided into 2 distinct types: retrotransposons, which use an RNA intermediate for transposition, and DNA transposons, which move only as DNA. Rapid advances in genome sequencing significantly improved our understanding of TE roles in genome shaping and restructuring, and studies of transcriptomes and epigenomes shed light on the previously unknown molecular mechanisms underlying genetic and epigenetic TE controls. Knowledge of these control systems may be important for better understanding of reticulate evolution and speciation in the context of bringing different genomes together by hybridization and perturbing the established regulatory balance by ploidy changes. See also sister article focusing on plants by Bento et al. in this themed issue.

The purpose of this CGR special issue is to outline the current trends in polyploidy research by drawing parallels between plant and animal polyploids and hybrids. However, when one considers the role of retrotransposons in polyploidy, this may not be such a trivial task. A simple PubMed search for co-occurring terms 'polyploid' and 'retrotransposon' retrieves exclusively publications involving plants, which are reviewed in this issue by Silva and colleagues [Bento et al., this issue]. There are several reasons which could be invoked to account for the paucity of literature on this subject in animals.

First, polyploidy is much less common in animals than in plants and has occurred in animals on relatively few occasions [Otto and Whitton, 2000; Soltis and Soltis, 2012]. For the most part, the reported cases of animal polyploidy, with a few exceptions, are ancient polyploids (paleopolyploids) which emerged relatively early in the course of evolution of selected lineages, such as tetrapods or teleost fish [Jaillon et al., 2009]. Moreover, because of the ancient nature of such events, it is no longer possible to determine whether they originated from a single progenitor species through chromosome doubling or fusion of unreduced gametes (autopolyploids) or from hybridization between 2 closely related species followed by genome doubling (allopolyploids), since the progenitor

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species are already extinct. The term whole genome duplication (WGD) is applicable to both auto- and allopolyploids.

Second, retrotransposons are generally more abundant in plant genomes than they are in animal genomes, often making up to 75% of plant genomic DNA [Schnable et al., 2009]. In many plant genomes, it is mostly LTR retrotransposons that serve as the major contributing factor to genome expansion/contraction [Vitte and Panaud, 2005]. Differential accumulation of retrotransposons, along with polyploidy, is one of the most-cited reasons for explaining the C-value paradox [Thomas, 1971], i.e. the lack of correlation between nuclear genome size and organismal complexity.

Third, the techniques for creation of synthetic polyploids, which allows researchers to trace genomic restructuring in real time with progenitor species also available for comparison, rather than to infer it from the molecular 'fossil record' with no clear understanding of the progenitors' genome structure, are much better developed in plants than in animals [Song et al., 1995; Matzke et al., 1999]. Since TEs are one of the most fluid components of the genome, the knowledge of TEs originally present in the progenitor(s) is highly relevant to understanding the nature of changes that occur as a result of ploidy increase, whereas the ancestral TE complements are much more difficult to infer than the ancestral states of protein-coding genes. Synthetic polyploids allow researchers to score any changes which occur prior to fixation or loss, even deleterious ones, while paleopolyploid analysis is confined to fixed changes.

Increasingly, TEs are being uncovered and catalogued in the course of genome sequencing projects, and when complex genomes are chosen for sequencing, the choice usually favors organisms with the least ploidy, so as to reduce sequencing costs and minimize assembly challenges. An example is provided by the clawed frogs: while the pseudotetraploid African clawed frog *Xenopus laevis* traditionally served as the favorite experimental object for embryologists for decades, nevertheless its less-studied diploid relative, the western clawed frog *Xenopus (Silurana) tropicalis*, was chosen for whole-genome sequencing as the first amphibian representative [Hellsten et al., 2010]. In case of ancient polyploidy, however, there is no such choice, and thus we know a lot more about genome structure in paleopolyploids than in neopolyploids. Overall, while genomic data on polyploid plants, in particular angiosperms, are increasingly becoming available due in part to their agricultural importance, there is still precious little genome sequence information on polyploid animals. In fact, out of more than a hundred polyploid animal species listed in Otto and Whitton [2000], Avise [2008] or Schön et al. [2009], none intersect with the list of sequenced genomes at the NCBI as of March 2013, with the exception of ancient polyploids such as tetrapods and teleost fish.

In light of the above information, it may be asked whether there is sufficient data in the literature to provide a comprehensive overview of retrotransposon behavior in polyploid animals. We will therefore try to add information on animal DNA transposons wherever relevant and, in some cases, will also mention fungi and protists in order to draw parallels between diverse eukaryotic systems. We hope that our efforts will help to stimulate further research in this area, especially the much-needed high-quality genomic and transcriptomic studies of hybrid and polyploid animals.

Major Types and Distribution of (Retro)transposons in Animals

TEs are discrete genetic units that have the ability to change their location within chromosomal DNA, thereby constituting the most flexible parts of the genome. They are broadly divided into 2 distinct types: retrotransposons and DNA transposons. Retrotransposons use an RNA intermediate for transposition, while DNA transposons move only as DNA. TE structure and genomic impact have been described in numerous reviews [e.g. Feschotte and Pritham, 2007; Jurka et al., 2007], and here we will briefly summarize only those features that are relevant to understanding of the following sections.

All autonomous retrotransposons code for reverse transcriptase, an enzyme responsible for RNA-directed DNA synthesis, which converts the retrotransposon transcript into a cDNA copy. Integration of this cDNA into new genomic locations depends on the element-encoded endonuclease (represented by integrase (IN), tyrosine recombinase (YR), or AP-, REL- or GIY-YIG-endonuclease). In principle, this 'copy-and-paste' process may result in formation of multiple new copies from multiple transcripts originating from the same genomic copy. Several retrotransposon classes can be distinguished on the basis of overall structure and mode of replication, namely: LTR retrotransposons flanked by long terminal repeats (LTRs) which are structurally similar to retroviruses; non-LTR retrotransposons with no terminal repeats; and *Penelope*-like elements (PLEs) with either direct or inverted repeats at the flanks (fig. 1) [reviewed in Havecker

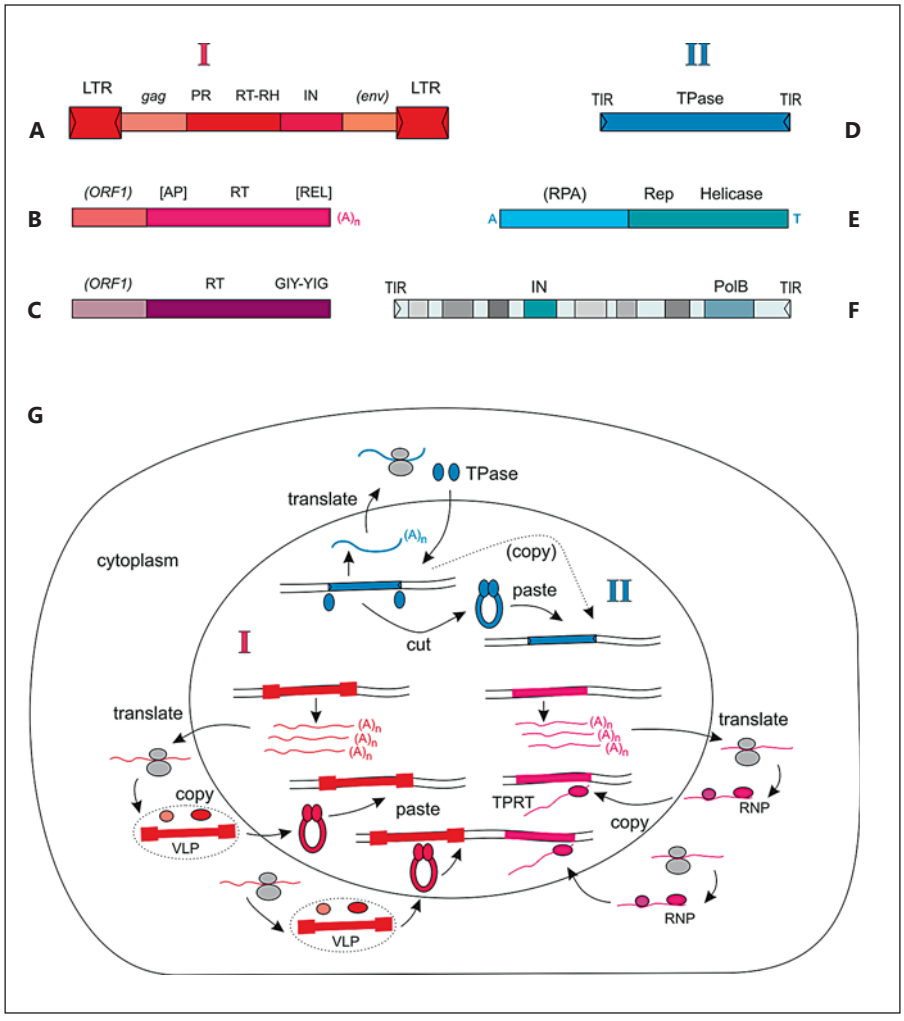

Fig. 1. Structural organization and replication cycles of the major eukaryotic TE classes: I, retrotransposons (red); II, DNA transposons (blue). **A** LTR retrotransposons; **B** non-LTR retrotransposons; **C** *Penelope*-like elements (PLEs); **D** cut-and-paste DNA TEs; **E** Helitrons; **F** Polintons/Mavericks; **G** TE replication cycles [after Eickbush and Malik, 2002; Havecker et al., 2004; Arkhipova, 2006; Kapitonov and Jurka, 2006, 2007; Jurka et al., 2007; Pritham et al., 2007]. $(A)_n$ = poly(A) sequence; A, T = nucleotides at the insertion site; IN = integrase; LTR = long terminal repeat; PolB = DNA polymerase B; PR = protease; Rep = rolling-circle replication protein; RH = RNase H; RNP = ribonucleoprotein; RPA = related to replication protein A; RT = reverse transcriptase; TIR = terminal inverted repeat; TPase = transposase; TPRT = target-primed reverse transcription; VLP = virus-like particle. TE-encoded products are shown by colored ovals, ribosomes by gray ovals. Optional open reading frames (ORFs) are shown in parentheses; non-LTR retrotransposon RT may contain an N-terminal AP-endonuclease or a C-terminal REL-endonuclease domain, and PLE RT may contain a C-terminal GIY-YIG endonuclease domain. Not to scale.

et al., 2004; Evgen'ev and Arkhipova, 2005; Arkhipova, 2006; Han, 2010]. While retrotransposons flanked by direct repeats can excise from the genome via LTR-LTR recombination, leaving behind a solo LTR, retrotransposons without terminal repeats do not have a specific mechanism for excision and can remain at their insertion sites for extended periods of time, eventually accumulating nucleotide substitutions and indels. Retrotransposons are further subdivided into different clades, based on the type of associated endonuclease, terminal structures and phylogenetic history [Eickbush and Malik, 2002].

DNA TEs can be subdivided into canonical 'cut-and-paste' transposase-encoding TEs, currently comprising 20 superfamilies, and replicative TEs such as rolling-circle Helitrons and self-synthesizing Polintons/Mavericks, coding for helicase and PolB-type polymerase, respectively [Feschotte and Pritham, 2007; Jurka et al., 2007; Kapitonov and Jurka, 2008]. Cut-and-paste DNA TEs are flanked by terminal inverted repeats (TIRs) and are capable of active excision, which for some superfamilies can be precise. Replicative TEs do not have the ability to excise and accordingly have a somewhat larger proliferative potential. The genome-wide dominance of retrotransposons is typically assumed by default, although DNA TEs may occasionally outnumber retrotransposons in selected genomes, as for example in *Caenorhabditis elegans* or *X. tropicalis* (fig. 2). In terms of total length, retrotransposons also have the upper edge, as they are generally longer than DNA TEs. Finally, both DNA TEs and LTR retrotransposons are more prone to horizontal transfers than non-LTR retrotransposons because of the presence of stable double-stranded DNA intermediates in their replication cycle [Eickbush and Malik, 2002]. On balance, in the majority of studied animal genomes, retrotranspo-

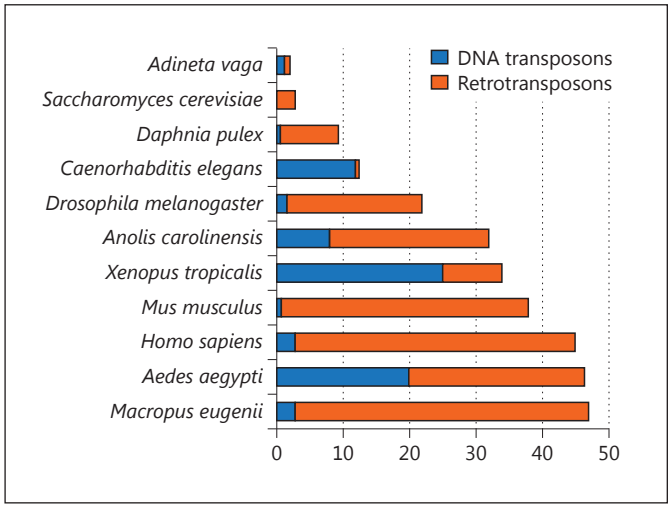

Fig. 2. Relative TE content in sequenced genomes of selected animal species described in the text. For comparison, the canonical model organisms *S. cerevisiae, C. elegans, D. melanogaster*, *M. musculus* and *H. sapiens* are included. The x-axis shows the percentage of the genome assembly occupied by each TE class, as compiled from the literature [International Human Genome Sequencing Consortium, 2001; Mouse Genome Sequencing Consortium, 2002; Rizzon et al., 2002; Kapitonov and Jurka, 2003; Kazazian, 2004; Nene et al., 2007; Hellsten et al., 2010; Alföldi et al., 2011; Colbourne et al., 2011; Renfree et al., 2011; Flot et al., 2013].

sons dominate because of their larger unit length, lower excision capacity and higher replicative potential, although there are of course exceptions to this rule.

Genetic and Epigenetic Controls of (Retro)transposon Mobility

In this section, we will summarize the major genetic and epigenetic mechanisms which are used to keep eukaryotic TEs under control [Charlesworth et al., 1994; Arkhipova and Meselson, 2005; Levin and Moran, 2011; Fedoroff, 2012]. Copy number control results from both host-based and TE-based controls of transposition, as well as from natural selection against deleterious effects of TEs, such as ectopic recombination and insertional mutagenesis. High transposition rates are usually counteracted by excision, mutational decay and selection against TEs, and can be controlled via element-based or host-based mechanisms. Element-based self-limitation arises when all members of a transposon family share a *trans*-acting component of the transposition machinery or are responsive to an element-encoded *trans*-acting repressor. It may include competitive inhibition, dominant-negative complementation, overproduction inhibition, and element-based repression [Hartl et al., 1997]. Self-limitation is characteristic of DNA TEs, in which transposase usually acts in *trans*, but does not typically develop in retrotransposons, in which reverse transcriptase strongly favors *cis*-action over *trans,* and any self-limiting elements will lose out to their non-self-limiting competitors [Arkhipova and Meselson, 2005].

Host-based suppressors can be represented by protein-coding genes which negatively affect transposition of specific TEs or certain types of TEs. Such genes would typically have other functions in the cell besides suppression of TE mobility. Several systems, however, are thought to have originally evolved to suppress TE activity. Examples are provided by cytosine deaminases such as APOBEC3, as well as C5-cytosine DNA methyltransferases (DNMT) [Yoder et al., 1997; Chiu and Greene, 2008]. APOBEC3 enzymes in mammals act on single-stranded DNA (ssDNA), and their activity can result in suppression and hypermutation of TEs having ssDNA in their replication cycle, such as retrotransposons [Carmi et al., 2011]. Genomic DNA methylation by DNMTs initially silences TEs epigenetically and over time leads to accumulation of C→T transitions due to deamination of 5-methylcytosine, which directly impacts TE coding potential. However, quite a few invertebrate taxa, for example *C. elegans*, do not exhibit DNA methylation and lack the corresponding enzymes altogether [Bird et al., 1995; Gutierrez and Sommer, 2004].

Besides DNA methylation, epigenetic control of TE mobility can occur at the level of small RNA-mediated silencing and chromatin remodeling via covalent histone modifications. The molecular underpinnings of small RNA-mediated epigenetic TE control systems in animals are briefly summarized in the next section.

Hybrid Dysgenesis and Mechanisms of Retrotransposon Silencing in Intraspecific Crosses

The phenomenon of hybrid dysgenesis, observed in certain interstrain crosses, refers to dramatic reduction in fertility, which is usually accompanied by activation of one or more TE families. Therefore, this section describes intraspecific rather than interspecific hybridization. While the underlying molecular mechanisms may not always be directly applicable to interspecific hybrids, knowledge of these mechanisms is essential to our under-

standing of epigenetic TE controls, which are often regarded as an adaptive immune system against TEs.

Originally, hybrid dysgenesis was described in *Drosophila melanogaster* in 2 separate systems, the P-M and I-R system. In the P-M system, most of the *D. melanogaster* strains can be classified in 2 categories, M (maternal) and P (paternal) strains. If females from an M strain are crossed with males from a P strain, the F1 progeny exhibits dysgenic traits such as reduced fertility and viability, gonadal atrophy, chromosomal aberrations, and a high rate of spontaneous mutation, while the progeny of the reciprocal cross does not show any abnormalities [Kidwell et al., 1977]. In the I-R system, hybrid dysgenesis is manifested when I (inducer) males are crossed with R (reactive) females: the female progeny develops normal ovaries and lays normal amounts of eggs, but the resulting embryos fail to hatch and display catastrophic meiosis [Picard and L'Héritier, 1971; Orsi et al., 2010].

It took nearly a decade to establish a link between hybrid dysgenesis and TEs. The discovery that hybrid dysgenesis in *D. melanogaster* can be caused by the P element, a DNA TE [Bingham et al., 1982], and by the I element, a non-LTR retrotransposon [Bucheton et al., 1984], shed the first light on its molecular basis. Subsequent studies provided new examples of hybrid dysgenesis, such as H-E dysgenesis in *D. melanogaster,* caused by *hobo* DNA TE [Blackman et al., 1987], and hybrid dysgenesis in *D. virilis*, where several TE families, mostly retrotransposons, are mobilized in a dysgenic cross [Petrov et al., 1995; Evgen'ev et al., 1997; Vieira et al., 1998]. Hybrid dysgenesis-like phenomena have been observed in interstrain crosses in other insects, such as the midge *Chironomus thummi* [Hägele and Oschmann, 1987] and the medfly *Ceratitis capitata* [Torti et al., 1994], although mobilization of specific TEs was not demonstrated directly.

Another 2 decades had to pass before hybrid dysgenesis became associated with host epigenetic TE control systems, specifically RNA-mediated silencing, which relies on production of small RNA molecules leading to transcriptional or post-transcriptional gene silencing. Among the different classes of endogenous small RNAs, microRNAs (miRNAs) interfere with mRNA translation, while small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs) can both be linked to TE suppression [Aravin et al., 2007b; Lau, 2008; Ghildiyal and Zamore, 2009; Siomi et al., 2011]. siRNAs, which are 20–24 nt long and are derived from a double-stranded RNA (dsRNA) precursor, are involved in antiviral defense response and in silencing of endogenous TEs in somatic cells [Czech et al., 2008; Ghildiyal et al., 2008; Saleh et al., 2009]. piRNAs interact with Piwi proteins, members of the Argonaute protein family, and control TEs in the germline of diverse metazoan species [Vagin et al., 2006; Aravin et al., 2007a, b; Brennecke et al., 2007; Siomi et al., 2008, 2011]. piRNAs are typically 24–31 nt long and are presumed to be derived from long RNA transcripts that are predominantly antisense to TEs. While biogenesis of miRNA and siRNA from dsRNA precursors depends on the type III ribonuclease Dicer, piRNA biogenesis is Dicer-independent. Different mutants in the piRNA pathway can be associated with different types of derepressed TEs [Vagin et al., 2006; Chen et al., 2007; Chambeyron et al., 2008]. Malfunction of epigenetic controls results in transcriptional activation and mobilization of TEs in *Drosophila* dysgenic hybrids and can also involve changes in chromatin structure and modification [Blumenstiel and Hartl, 2005; Girard and Hannon, 2008; Rozhkov et al., 2010, 2013].

In the I-R system, the I-element is abundant but silenced in the euchromatin of the I strains of *D. melanogaster*, while it is absent from the R strains. As the directionality of inter-strain crosses is crucial for manifestation of dysgenic traits in *Drosophila*, it has been proposed that piRNAs represent the transmissible agents that can be passed on to the progeny via the maternal germline to yield TE repression [Brennecke et al., 2008]. This maternal protection is reduced in R strains, permitting paternally transmitted I-elements to become derepressed in the germline of hybrid females [Brennecke et al., 2008; Chambeyron et al., 2008]. In addition to small RNAs, TE control in the germ cells depends on the Piwi family proteins (Piwi, Aubergine and Argonaute3 in *Drosophila*), which play an important role in TE silencing by binding piRNAs and using them as guides for RNA slicing activity. Piwi proteins are deposited into developing oocytes [Cox et al., 1998], providing a basis for maternal transmission of the Piwi-piRNA complex to the offspring. In general, Piwi proteins are important for gonadal development in metazoans, and piRNA-interacting protein mutants in *Drosophila* and mouse exhibit TE derepression in the germline [Vagin et al., 2004; Aravin et al., 2007b; Li et al., 2009; Juliano et al., 2011; reviewed in Siomi et al., 2011].

In *D. virilis,* hybrid dysgenesis occurs when females of an M-like laboratory strain 9 are mated with males of a P-like strain 160. In these crosses, most of the progeny (male and female) are sterile, while the reciprocal cross yields normal progeny [Lozovskaya et al., 1990]. Diverse TE families, including both retrotransposons and DNA

TEs, are mobilized, although the retrotransposon *Penelope* seems to play an important role [Evgen'ev et al., 1997; Vieira et al., 1998]. In this system, *Penelope* is abundant in *D. virilis* strain 160 and is entirely absent from strain 9. Initially, maternally deposited siRNAs homologous to *Penelope* were reported in P-like strains but were absent from M-like strains [Blumenstiel and Hartl, 2005]. A more thorough analysis of small RNAs in *D. virilis* was performed by Rozhkov et al. [2010, 2013], where *Penelope* was shown to be targeted by antisense siRNAs, and only a small proportion represented the 23–29-nt piRNA fraction, which could be related to germline silencing. The introduction of active *Penelope* into *D. melanogaster*, which has no preexisting *Penelope* copies in the genome, caused induction of both sense and antisense siRNAs in the transformed strains, suggesting that the siRNA pathway could play an important role at the initial stages of TE invasion, acting similar to an antiviral response. Following *Penelope* mobilization and amplification, its copies can insert into piRNA clusters, triggering production of piRNAs to maintain silencing in the germline [Rozhkov et al., 2013].

The piRNA clusters represent discrete loci in the genome, which consist mostly of TE remnants giving rise to piRNA precursor transcripts. The *flamenco* piRNA cluster was originally identified as a locus controlling the activity of *gypsy*, *Idefix* and *ZAM* retrotransposons in *D. melanogaster* [Pelisson et al., 1994; Desset et al., 2003]. While *flamenco* represents an example of a strand-biased cluster, in which TEs are mostly found in the same orientation and could yield a single precursor transcript, there are also dual-strand piRNA-producing clusters, which contain TE insertions in both orientations. The RNP complexes formed by Piwi and Aubergine proteins preferentially incorporate the primary antisense TE strand, while the Argonaute3 complex carries secondary sense piRNAs [Brennecke et al., 2007]. Together, they are presumed to perpetuate the so-called ping-pong amplification loop, in which primary piRNAs direct cleavage and production of secondary piRNAs, thereby continually targeting active TE transcripts [reviewed in Juliano et al., 2011; Siomi et al., 2011]. While the piRNA clusters constitute the genetic memory recording past TE invasions in the genome that can provide immunity to subsequent invasions of the same TE, recent studies also point at the existence of epigenetic memory, mediated by trans-generational inheritance of piRNAs corresponding to non-self single-copy inserts [Ashe et al., 2012; Lee et al., 2012; Shirayama et al., 2012]. Such piRNAs could be particularly helpful in repressing TEs at the initial stages of invasion.

Although the first piRNAs were described in mammals [Aravin et al., 2006; Girard et al., 2006; Grivna et al., 2006], in which TE suppression occurs but does not appear to be their primary role, their identification was subsequently extended to virtually all animals studied, depending mostly on the availability of sequenced genomes and small RNA transcriptomes (human, mouse, rat, zebrafish, platypus, insects, worms, sponges, etc.) [Aravin et al., 2007b; Das et al., 2008; Grimson et al., 2008; Houwing et al., 2008; Murchison et al., 2008]. In plants, piRNAs and Piwi proteins are lacking, although their function to a certain extent is performed by siRNAs [Cantu et al., 2010; Martienssen, 2010]. In summary, small RNA molecules are increasingly emerging as major players in situations when different genomes need to be scanned against each other.

Retrotransposons in Interspecific Hybrids

In general, the union of 2 divergent genomes caused by hybridization leads to multiple consequences, including but not limited to changes in gene expression and DNA methylation, chromosomal rearrangements and TE mobilization. Interspecific hybridization and the corresponding 'genomic shock' resulting from it were suggested to induce bursts of transposition by McClintock [1984]. Without attempting to review the vast literature on interspecific hybridization and the emergence of reproductive barriers leading to speciation, we will briefly review the information available with regard to retrotransposon behavior in interspecific crosses, noting that these examples represent diploids rather than polyploids.

Transposition rates of the LTR retrotransposon *Osvaldo* were increased by an order of magnitude in introgressed hybrids between *Drosophila buzzattii* and *D. koepferae* when compared to non-hybrids [Labrador et al., 1999; Fontdevila, 2005]. Retrotransposons could have been involved in generation of the so-called 'evolutionary' breakpoints in the *virilis* species group of *Drosophila*: the euchromatic insertion sites, as well as the breakpoints of rearrangements caused by retroelements *Penelope* and *Ulysses* in the progeny of dysgenic crosses in *D. virilis,* often coincide with breakpoints of inversions previously established for other species of the *virilis* group [Zelentsova et al., 1999; Evgen'ev et al., 2000]. In natural populations of *D. buzzattii*, polymorphic chromosomal inversions were shown to originate via ectopic recombination between copies of a DNA TE *Galileo*, which along with re-

lated elements inhabits the *buzzattii* species complex and is thought to have played a role in chromosomal evolution and speciation of the complex [Cáceres et al., 1999; Casals et al., 2005; Delprat et al., 2009].

It is tempting to hypothesize that the breakdown of epigenetic controls known to occur during hybrid dysgenesis in intraspecific crosses can be extended to interspecific hybrids in order to understand the basis of their incompatibility. A recent study by Kelleher et al. [2012] attempted to study differences in small RNA production in interspecific crosses between *D. melanogaster* and *D. simulans*. However, the results differed significantly from the expectation that the lack of maternally deposited piRNAs would result in activation of incoming TEs provided by the paternal genome. Instead, derepression of both paternally and maternally inherited TE families was observed, whereby the most derepressed TEs corresponded to the most active and recently arrived families. The observed defects in piRNA production phenocopied (i.e. yielded phenotypes identical to) the known mutations in several piRNA effector proteins, pointing at general dysfunction of the piRNA-producing machinery rather than the more specific response to the presence/absence of certain TEs in the maternally transmitted piRNA pool. While it was not possible to attribute the hybrid phenotype to any specific piRNA effector protein, several such proteins were previously shown to undergo adaptive evolution, leading to their rapid divergence [Vermaak et al., 2005; Obbard et al., 2009]. It is not yet clear what exactly is driving such adaptive evolution, as the interaction between TE-derived RNAs and piRNA effector proteins is not sequence-specific. Nevertheless, at least one of such genes, *D. simulans aubergine*, was not able to fully complement its *D. melanogaster* counterpart, indicating mild incompatibility [Kelleher et al., 2012].

Retrotransposon activation has also been reported in interspecific mammalian hybrids. In marsupials, such hybrids exhibit genome-wide demethylation, chromosome rearrangements and amplification of mobile elements [O'Neill et al., 1998; Brown and O'Neill, 2010]. Crosses between 2 kangaroo species, *Macropus eugenii* (tammar wallaby) and *Wallabia bicolo*r (swamp wallaby), produce hybrids exhibiting dramatic genome-wide undermethylation. This can lead to derepression of TEs, the activity of which is epigenetically controlled by DNA methylation. One of the consequences of this activity is the appearance of atypical extended centromeres in autosomes from the *M. eugenii*-derived subgenome, caused in part by accumulation of KERV-1 (kangaroo endogenous retrovirus 1) in the centromere region. Hybrids between other marsupial species yield de novo chromosome rearrangements with discrete breakpoints in the karyotype within one generation, resulting in sterile progeny [O'Neill et al., 2001].

Among eutherian mammals, dysgenic effects in interspecific hybrids, such as male sterility, abnormal growth and defects in placenta, were reported in *Mus musculus* × *M. spretus* hybrids [Schütt et al., 2003]. Important epigenetic variations could be localized to the X chromosome, where hypomethylation was found in genes adjacent to endogenous retrotransposons LINE-1 and IAP. In *M. musculus* × *M. caroli* hybrids, fetuses are rarely carried to term, owing to placental dysplasia. The placentas developed in this cross via artificial insemination, despite having a normal karyotype, displayed an anomalous expression of methyltransferases that could be associated with methylation perturbations targeting retrotransposons and with double minute chromosome formation [Brown et al., 2008, 2012]. The placental abnormalities in mouse interspecific hybrids could result from imbalance in DNA methylation as a defense mechanism against genome instability resulting from deregulation of retroelements.

Retrotransposons were also involved in centromeric expansion and differential methylation in gibbons, apes of the family Hylobatidae. Gibbons are a large taxonomic group with 4 genera and 17 described species, which have an unusually high rate of chromosome rearrangements [Cunningham and Mootnick, 2009]. In northern white-cheeked gibbons *(Nomascus leucogenys)*, the 'evolutionary breakpoints' corresponding to rearrangements between gibbon and human chromosomes were associated with hypomethylated *Alu* retrotransposons, suggesting that changes in the epigenetic state of *Alu* repeats could enhance genome restructuring [Carbone et al., 2009]. In the eastern hoolock gibbon *(Hoolock leuconedys)*, a burst of retrotransposition, associated with expansion of most centromeres, was produced by a novel gibbon-specific composite retroelement called LAVA, formed by portions of 3 other retrotransposons (L1, AluS, and SVA), which could have been activated by hypomethylation [Carbone et al., 2012]. This scenario resembles the expansion of KERV-1 in centromeres of the wallaby [O'Neill et al., 1998; Ferreri et al., 2011].

In plants, retrotransposon amplification and/or epigenetic alteration was observed in interspecific hybrids of diploid as well as polyploid species, especially in pericentromeric regions [reviewed in Michalak, 2009, 2010; Feldman and Levy, 2012]. Reduction in siRNA and methylation levels was directly linked to retrotransposon activation in polyploid wheat hybrids, invoking similarity with

hybrid dysgenesis in *Drosophila* [Kenan-Eichler et al., 2011]. Interploid and interspecific crosses in *Arabidopsis* and the emerging post-fertilization barriers are well-correlated with the breakdown of siRNA-mediated TE epigenetic controls leading to a 'genomic shock', and their similarity with hybrid dysgenesis systems was also underscored [Ha et al., 2009; Martienssen, 2010]. Overall, while the scarcity of data for animal hybrids and polyploids precludes broad generalizations, it would be hard to disregard the potential importance of TE contributions to genome-wide changes in hybrid and polyploid species.

Pseudotetraploidy and Reversion to Stable Diploidy

After polyploidization or WGD, the polyploid genome gradually reverts to its original diploid state in a process called diploidization [Wolfe, 2001], restoring the ability of chromosomes to pair exclusively with their homologs and not with homeologs which arose as a result of WGD. This can be commonly achieved through differential loss of selected genes in former homologs [reviewed in Semon and Wolfe, 2007]. Those duplicated genes that have not undergone deletion can undergo nonfunctionalization, neofunctionalization or subfunctionalization, whereby they are subject to inactivation, evolution towards a different function for one of the duplicates or towards complementary function for both duplicates, respectively [Lynch and Conery, 2000]. Together with chromosomal rearrangements such as deletions, inversions and translocations, these changes would constitute the genetic response to WGD leading to diploidization, which may result in emergence of distinct populations that are substantially different to become reproductively isolated.

Activation of certain retrotransposons following polyploidization has been reported in selected plant species, such as wheat and tobacco, in terms of either transcriptional activation or copy number increase [Parisod et al., 2010]. Since these studies mostly targeted the known active retrotransposon families, possible activation of other, yet unknown families may have gone undetected. Overall, TE amplification appears to be less general than massive TE losses via recombination-mediated deletions, as well as epigenetic changes [Parisod et al., 2010]. For animals, the relevant data are largely missing, and detailed comparison of genomes differing by ploidy levels has not yet been performed on a genome-wide level. Polyploidization in animals could potentially cause retrotransposon activation similar to that observed in plants and could be associated with epigenetic changes. Copy numbers may also undergo increase due to relaxation of selective pressures on TE mobility that would normally limit the number of deleterious insertional mutations, including doubling of the number of available insertion targets with extra copies of genes to compensate for the damaged ones [Matzke and Matzke, 1998].

In general, TE activation upon polyploidization would not necessarily be manifested in the form of amplification, i.e. substantial increase in copy number, although for retrotransposons transcriptional activation represents a necessary precondition for amplification. Even without a dramatic copy number increase, a certain level of TE activation may lead to chromosomal rearrangements such as deletions, inversions or translocations, all of which could stimulate diploidization by reducing the degree of collinearity between homeologous chromosomes. Active chromosomal rearrangements mediated by transposase activity of DNA TEs, or brought about by polymerases during replication of inverted-repeat TEs, are particularly likely to contribute to rapid genome reshaping in the absence of large-scale TE amplification [Gray, 2000; Mizuno et al., 2013].

TEs in Selected Polyploid Animal Taxa

It is not necessary to review here the distribution and behavior of retrotransposons in genomes that underwent ancient polyploidization and since then evolved for hundreds of millions of years in rediploidized state, as it would be difficult to imagine how these genomes would have developed without undergoing WGD, as well as the impact it would have had on their TE content, without any information on their progenitors. No viable neopolyploids are known in mammals, in which polyploidy is usually associated with fatal developmental abnormalities and cancer, except for a controversial case of the allotetraploid red viscacha rat *Tympanoctomys barrerae* [Gallardo et al., 2006; Suárez-Villota et al., 2012; but see Svartman et al., 2005]. It is of interest, however, to consider selected non-mammalian species, especially those in which ploidy changes are superimposed on the asexual mode of reproduction, although it would be challenging to tease apart the relative contribution of these factors. Indeed, asexual reproduction is unknown in mammals, and about one-third of polyploid animals are asexual [Otto and Whitton, 2000], a disproportionally high number with regard to overall polyploidy incidence in animals. Below we consider several examples of paleopolyploids and neopolyploids, both sexual and asexual, for

which at least some genomic and transcriptomic information regarding TE composition and behavior is available. It may be expected that more substantial progress in these systems will be accomplished in the near future.

Rotifers

Bdelloid rotifers are microscopic freshwater invertebrates best known for their long-term asexuality, the ability to undergo frequent cycles of desiccation and rehydration and to resist ionizing radiation, and the propensity for horizontal gene transfer [Mark Welch and Meselson, 2000; Gladyshev and Meselson, 2008; Gladyshev et al., 2008]. Molecular studies of long stretches of bdelloid DNA such as cosmid and fosmid clones revealed that bdelloids are degenerate tetraploids, with the origin of tetraploidy predating the divergence of the major bdelloid families [Mark Welch et al., 2008, 2009; Hur et al., 2009]. The observed quartet structure is highly reminiscent of that in paleotetraploid yeast or *Tetraodon* genomes [Jaillon et al., 2009], whereby the progenitor genome(s) after a WGD undergoes numerous segmental deletions that involve one or the other member of a homeologous pair, over evolutionary time resulting in preservation of relatively few genes that are common to both homeologs.

Those bdelloid gene-rich regions in which synteny can be reliably traced are exceptionally low in TE content, although the high degree of DNA turnover leaves insufficient 'fossil record' in genomic DNA to determine whether the ancestor of modern bdelloids had a substantial intergenic TE content before or shortly after the WGD event. This applies both to earlier studies of cosmid/fosmid clones [Gladyshev and Arkhipova, 2007, 2009a, b, 2010a] and to recent more comprehensive analyses performed on a genome-wide level [Flot et al., 2013]. Thus, it is difficult to tell whether polyploidization in bdelloids was followed, in addition to genic deletions, by extensive deletion of TE sequences located in intergenic regions, although such possibility appears likely. Also, TE proliferation may have subsided following loss of sex, due to the fact that the ability of TE insertions to go to fixation in sexually reproducing populations, even if they are deleterious, does not apply to apomictic populations [Hickey, 1982; Arkhipova and Meselson, 2005]. In any case, at least in the genome of the sequenced bdelloid representative *Adineta vaga*, the end result is the near-elimination of TEs from gene-rich regions and the overall low TE content of about 3% [Flot et al., 2013].

Known retrotransposons in *A. vaga* occupy a relatively small fraction of the genome, making up slightly over 1%, which is less than the fraction occupied by DNA TEs. In terms of family diversity, DNA TE families outnumber retrotransposons more than 2-fold, and include 14 of 20 known superfamilies [Arkhipova and Meselson, 2005; Gladyshev and Arkhipova, 2009a; Flot et al., 2013]. Nevertheless, retroelement diversity is also quite high, with 4 clades of LTR retrotransposons, 6 clades of non-LTR retrotransposons, and 2 clades of PLEs [Gladyshev et al., 2007; Gladyshev and Arkhipova, 2007, 2009b; Flot et al., 2013]. The prevalence of DNA TEs may be explained by their propensity for horizontal transfer, which is also applicable to LTR retrotransposons. The intactness of most copies and the high degree of identity between LTRs also argue in favor of recent arrivals, which, however, do not lead to TE amplification: most families are represented by only 1 or 2 intact copies and, on average, about 10 times as many fragmented copies. Many defective copies contain microhomology-mediated deletions, which could result from imprecise repair of double-strand breaks following repeated cycles of desiccation and rehydration [Gladyshev and Arkhipova, 2010b; Flot et al., 2013]. Overall, while it cannot be ruled out that ancient WGD was associated with short-term TE amplification, it appears that, in the long term, TE copy number was brought down by combined effects of asexual lifestyle, mutational inactivation and efficient silencing mechanisms. Indeed, *A. vaga* possesses a highly diversified RNA-mediated silencing machinery, including 23 different Argonaute/Piwi family proteins [Flot et al., 2013].

Arthropods

Although researchers for a long time have been taking advantage of polyploidy in certain insect tissues, such as polytene chromosomes in *Drosophila* larval salivary glands, ploidy changes in insects at the organismal level mostly involve numerous haplodiploid species in the order Hymenoptera (ants, bees, sawflies, and wasps), in which males are haploid and females diploid [Normark, 2003]. In crustaceans, however, polyploidy occurs at the organismal level: in the aquatic microcrustacean *Daphnia*, occasional polyploidy is thought to arise from interspecific hybridization, leading to emergence of asexual lineages [Mergeay et al., 2008; Vergilino et al., 2009, 2013]. The first sequenced crustacean genome is that of the diploid *Daphnia pulex* [Colbourne et al., 2011]. TEs constitute 9.4% of its genome assembly, and retrotransposons strongly predominate (fig. 2). DNA TEs constitute only 0.7% of the genome and are represented by 56 families from 10 superfamilies. Non-LTR retrotransposons belong to 5 clades and 78 families, while LTR ret-

rotransposons are the most numerous, both in terms of family number (141 families) and cumulative length (nearly 8% of the genome). Earlier studies [Valizadeh and Crease, 2008; Rho et al., 2010; Schaack et al., 2010a, b] attempted to compare activity of various TEs in obligately asexual and cyclically parthenogenetic *Daphnia* populations and were generally consistent with the idea that sexual reproduction facilitates TE spread [Hickey, 1982], although several opposing forces may be at play. The sequenced *D. pulex* genome provides a solid foundation for further investigations into genomic studies of its hybrid and polyploid relatives, including in-depth TE analyses.

Fish

While over 20 fish genomes have been sequenced to varying degrees of completion, comparative genomics of neopolyploid fish such as salmonids or cyprinids is still in its infancy [Davidson et al., 2010; Bernardi et al., 2012]. In salmonids, a family comprising about 30 species, WGD is thought to have occurred 25–100 Mya, and the process of diploidization is well underway. The Atlantic salmon *(Salmo salar)*, with a ~1-Gb genome in 29 chromosome pairs, was chosen for sequencing because of its economic importance. The 2 subgenomes within this pseudotetraploid species differ by ~10%, which should facilitate proper subgenome assembly. However, the high content of repeats, which make up about one-half of the genome, poses significant assembly challenges. Initial characterization of repeats in BAC clones revealed waves of recent expansion of Tc1-like DNA TEs, exhibiting correlations with the timing of salmonid radiation [de Boer et al., 2007]. The authors proposed that these TEs were introduced horizontally, as inferred from the higher degree of similarity between TEs from other species than between protein-coding genes from the corresponding species, and invoked parasites as possible vectors based on TE similarities to *Schistosoma* TEs. Similar conclusions were made regarding non-LTR retrotransposons of the L2 clade and their partner SINEs [Matveev and Okada, 2009]. With the rainbow trout *(Oncorhynchus mykiss)* genome project also underway, detailed genome-wide TE analyses are forthcoming and should reveal any TEs that are common to the genomes of salmonids and northern pike, their closest diploid relative.

None of the known fish species are true parthenogens. Many, however, reproduce by paternal leakage (whereby sperm contributes to unreduced ova) or hybridogenesis (hemiclonal reproduction where only maternal genome is transmitted), and these are often triploid or tetraploid, depending on the degree of sperm and egg contribution to the progeny [Lamatsch and Stöck, 2009]. Genomic studies of such hybrids have not yet been initiated, and therefore poeciliid, cyprinid, and cobitid fish would be particularly attractive targets for genome-wide surveys. In polyploid carp hybrids, a Tc1-like retrotransposon was shown to be mobilized [Liu et al., 2009]. In the cyprinid *Squalius alburnoides*, transcriptomic studies point at increased miRNA expression in triploids in comparison to mid-parent values, possibly reflecting restoration of dosage balance to the diploid level [Inácio et al., 2012]. It may be hoped that at least some polyploids will make it to the short list of the vertebrate Genome 10K initiative, which will target as many as 4,000 fish genomes for sequencing in the next 5 years [Genome10K Community of Scientists, 2009].

Amphibians

The clawed frogs *Xenopus/Silurana* are a unique group of vertebrate animals which exhibit a remarkable variation in ploidy ranging from 2 to 12 [Evans, 2008]. So far, genome sequence information is available only for 2 species, the ~1.5-Gb diploid *X. tropicalis* and the ~3-Gb pseudotetraploid *X. laevis,* with an approximately double chromosome number (2n = 20 vs. 2n = 36). Allotetraploidy in *X. laevis* is thought to date back to 30–40 Mya, but an autotetraploid origin still cannot be ruled out [Hellsten et al., 2007]. About 35% of the *X. tropicalis* genome consists of TEs, with an unusual prevalence of DNA TEs which make up a quarter of the genome [Hellsten et al., 2010]. Diverse non-LTR (CR1, L2, Rex1, L1, Tx1 clades), LTR (gypsy, copia, BEL, ERV I, ERV III), *Penelope*, and DIRS retrotransposons together make up only 9% of the genome, while DNA TEs come from 7 major superfamilies (hAT, Harbinger, mariner/Tc, piggyBac, Kolobok, Helitron, Polinton). The rate of DNA turnover in *X. tropicalis* is relatively low, providing a reasonably good TE fossil record. The first public release of *X. laevis* genome v6.0 just became available [James-Zorn et al., 2013], finally providing the opportunity for genome-wide *X. tropicalis*-*X. laevis* comparisons, which will undoubtedly follow shortly.

Various interspecific crosses between over 20 species of clawed frogs are possible and yield mostly sterile males and fertile females; the interspecific cross between 2 tetraploid species *X. laevis* × *X. muelleri* was studied most intensively with regard to gene expression [Malone et al., 2007; Malone and Michalak, 2008a, b]. Analysis of miRNAs in *X. laevis* × *X. muelleri* hybrids with the aid of microarrays showed global underexpression of miRNAs, including those considered to be testis-specific

[Michalak and Malone, 2008]. These effects could have resulted from divergence of miRNA pathway components, as recently observed in *Drosophila* [Kelleher et al., 2012].

The availability of the *X. laevis* genome should stimulate comparative studies of small RNA transcriptomes in both *X. laevis* and *X. tropicalis,* facilitated by knowledge of respective TE complements. Experimental data on both *X. tropicalis* and *X. laevis* small RNA populations are available [Armisen et al., 2009; Kirino et al., 2009; Lau et al., 2009; Faunes et al., 2012]; however, these data have been mapped only to the *X. tropicalis* genome and have not yet been analyzed in detail in the context of *X. laevis* genome sequence. Thus, in *X. tropicalis* small RNA libraries, the amount of small RNAs mapped to known TEs averaged about 20%, while in libraries from *X. laevis* matches to known TEs constituted only 2% [Lau et al., 2009]. This is apparently because *X. laevis* TEs have not yet been subjected to full inventory and may not exhibit sufficiently strong homology to *X. tropicalis* TEs (for instance, retrotransposon Tx1_Xt is 85% identical to Tx1_Xl). Viable interspecific hybrids between these 2 species have been reported [Burki, 1985], and it would be of substantial interest to compare small RNA populations in the hybrids and both parental species to see whether TE derepression can be observed, although it would be a challenge to tease apart the effects of hybridization and ploidy change in interploidy crosses.

A different group of amphibians, plethodontid salamanders, are famous for their huge genomes measuring up to 75 Gb in size, and among animals they are outsized only by lungfish [Gregory, 2012]. It is natural to assume that, as in plants, retrotransposon expansions may provide a substantial contribution to such genomic gigantism. Indeed, early studies revealed that an LTR retrotransposon *Hsr1* was present in as many as 10^6 copies per genome in *Hydromantes*, outnumbering even mammalian L1 elements [Marracci et al., 1996]. A recent survey of 6 plethodontid species with genome sizes ranging between 15 and 47 Gb, based on approximately 0.1–2% genome coverage from 454 pyrosequencing, indicated that Ty3/gypsy LTR retrotransposons could represent the major contributors to genome expansion, followed by non-LTR/L2, DIRS and ERV1 retrotransposons [Sun et al., 2012a]. Deletion rate, as determined by analysis of non-LTR retrotransposons, was shown to be much lower in salamanders than in *Xenopus*, apparently resulting in accumulation of large amounts of intergenic DNA [Sun et al., 2012b]. Although salamanders do not appear to form ploidy series like frogs, changes in ploidy can be observed during transitions to asexuality: in ambystomatid salamanders, asexual triploids, tetraploids, or even higher ploidy individuals can be produced in interspecific crosses [Mable, 2007; Neaves and Baumann, 2011]. Although the TE landscape in salamanders still remains underexplored, limited synteny comparisons with frogs, chicken and humans suggest that genome expansion occurred via addition of extra interstitial DNA rather than WGD [Voss et al., 2011], consistent with participation of retrotransposons in differential genome expansions. It may be thought that TE complements in parental species giving rise to asexual hybrids could differ significantly, which may create problems for the sexual process. Nevertheless, recombination between homeologs has been observed in the polyploid unisexual salamander *Ambystoma* and was hypothesized to be a source of genetic variation [Bi and Bogart, 2010]. Since TEs carry numerous regulatory elements, such variation could lead to diversification of gene expression patterns and rewiring of regulatory networks. A combination of genomic and transcriptomic studies could shed light on these issues.

Reptiles

There are no reported cases of polyploidy in turtles, crocodiles or snakes, and neither are these taxa known to give rise to asexual species. However, true parthenogenesis does occur in lizards, of which about 0.6% are parthenogenetic, and nearly all parthenogens are hybrids, with 40% of them being polyploids (triploids) [Kearney et al., 2009]. The genome of the first and so far the only sequenced lizard, the sexually reproducing diploid green anole *(Anolis carolinensis)*, is close to 2 Gb in size, with ~30% represented by TEs [Alföldi et al., 2011]. Most TEs are active and well-diversified, and non-LTR retrotransposons (L1, L2, CR1, RTE, R4 clades) strongly predominate, with LTR transposons comprising only a minor fraction of TEs [Janes et al., 2010]. Many DNA TEs are thought to originate from horizontal transfers [Novick et al., 2010, 2011]. In addition, non-LTR retrotransposons exhibit a higher rate of DNA loss than in mammals, indicating a relatively rapid DNA turnover [Tollis and Boissinot, 2011; Sun et al., 2012b].

A diversified and dynamic pattern of TE organization is more similar to fish and amphibians than to mammals and birds, and while it remains to be seen whether such repetitive landscape is also characteristic of other lizards, comparison with the draft Burmese python *(Python molurus bivittatus)* and copperhead snake *(Agkistrodon contortrix)* genome sequences reveals a somewhat similar relative proportion of TE types [Castoe et al., 2011]. Over-

all, non-LTR retrotransposons are prevalent in amniote genomes, with LTR retrotransposons taking the back stage, and it has been argued that CR1 retrotransposons dominated the genomic landscape in the ancestral amniote [Shedlock, 2006; Shedlock et al., 2007]. It remains to be seen what kinds of repetitive sequences are associated with formation of microchromosomes in certain reptiles, such as the early-branching tuatara *(Sphenodon punctatus)* [Wang et al., 2006; O'Meally et al., 2009; Miller et al., 2012].

A particularly exciting area of research may be opened by the availability of viable interspecific polyploid hybrids in lizards [Lutes et al., 2011]. Self-perpetuating clonal lineages of a tetraploid whiptail lizard were derived by fertilization of triploid oocytes from a parthenogenetic *Aspidoscelis exsanguis* with haploid sperm from *Aspidoscelis inornata*. By analogy to synthetic polyploids in plants, studies of such hybrids should provide an excellent opportunity to monitor genomic changes in real time rather than on the evolutionary scale.

Comparison with Protist and Fungal Systems

Despite the existence of polyploidy in the fungal kingdom, in-depth investigations of fungal polyploidy, including studies of ancient WGDs and synthetic polyploids, so far have been mostly performed in the classical model yeast *Saccharomyces* [Albertin and Marullo, 2012]. These yeasts have low TE content (~3%; fig. 2) and have lost the RNA-mediated silencing machinery, so that the involvement of retrotransposons in polyploid genome evolution appears to be limited to their role in ectopic recombination: for instance, in allotetraploid lager yeast *S. pastorianus*, Ty1 elements are localized near translocation breakpoints. Given the relative ease of fungal genome sequencing and assembly in comparison to metazoans, studies of genetically tractable polyploid fungi retaining the epigenetic TE control systems could be particularly helpful in discerning the relative contributions of different TE control mechanisms to the 'genomic shock'.

RNA-mediated silencing machinery in ciliated protozoans is quite diversified and comes together with nuclear dualism: ciliates have germline diploid (transcriptionally silent) as well as somatic polyploid (transcriptionally active) nuclei [Prescott, 2000]. These protozoans can afford to have in their diploid micronuclear genome literally tons of 'junk DNA' such as TEs (up to 95% of the germline genome), which nevertheless does not pose additional burden for gene expression: in the somatic macronuclear genome, chromosomes are fragmented and amplified to high copy number through amitotic divisions, with ploidy levels rising hundred- or even thousand-fold. Separation of germline and somatic genomes and subsequent somatic polyploidization allows these protists to use their transcription and translation machinery only on protein-coding genes, without wasting any resources on non-needed sequences. In ciliates, as in animals, piRNAs apparently act as transgenerational carriers of epigenetic information [Sontheimer, 2012]. Moreover, in different ciliates the highly diversified piRNA machinery can act to produce seemingly opposite effects: while scanRNAs in *Paramecium* and *Tetrahymena* are derived from the germline micronucleus and mark for elimination only sequences that are not present in the maternal macronucleus [Mochizuki et al., 2002], piRNAs in a distantly related *Oxytricha* are derived from the somatic macronucleus and mark the developing zygotic macronuclear sequences for retention [Fang et al., 2012]. The existence of 'protective' or 'antisilencing' piRNAs agrees with recent studies in *C. elegans,* where certain piRNAs act to prevent germline-specific genes from being silenced, and perhaps to mark sequences for proper segregation [Lee et al., 2012; Shirayama et al., 2012].

The germline micronuclear genome can also undergo conventional WGD events resulting in ploidy changes: genome sequencing of *Paramecium tetraurelia* revealed 3 rounds of WGD, with the oldest round dating back prior to divergence between *Paramecium* and *Tetrahymena*, and the most recent having occurred prior to formation of the *Paramecium aurelia* species complex comprising at least 15 sibling species, providing tentative links between WGD and speciation [Aury et al., 2006]. Although transcriptome studies reveal very low rates of subfunctionalization [Arnaiz et al., 2010], a high degree of diversification is observed in Piwi proteins, allowing them to assume diverse functional roles in silencing as well as programmed genome rearrangements involving TE removal [Bouhouche et al., 2011].

Conclusions and Future Directions

Polyploidy and hybridization, which often occur simultaneously, are often viewed as major forces in evolution and speciation, because of immediate effects on gene dosage and longer-term effects on gene function. However, their influence on non-genic sequences is often overlooked, while in fact it may be of substantial importance for genome function and evolution. TEs and their

control mechanisms could play an important role in reshaping chromosome structure and gene expression, and in creating Dobzhansky-Muller incompatibilities, whereby negative epistatic interactions between diverging loci could result in reproductive isolation and speciation [Michalak, 2009; Brown and O'Neill, 2010].

Due to their high amplification potential, retrotransposons usually represent prime suspects for rapid genome expansions, especially under conditions that may disrupt normal operation of the TE control systems. So far, the most convincing cases for retrotransposon activation and their involvement in hybrid incompatibility following interspecific crosses have been made in plants, and studies in the animal systems have been lagging behind. Notwithstanding the obvious differences between animals and plants, such as the lack of biparental cytoplasmic contribution to the animal zygote and the lack of sequestered germline in plants, there are still plenty of cases to be made for TE activation in animals under conditions of 'genomic stress' [McClintock, 1984]. In many animals and plants, LTR retrotransposons appear to be a dominant force in genome expansion, which is probably fueled both by their high replicative potential as well as the propensity for horizontal transmission, although in amniotes non-LTR retrotransposons begin to dominate the genomes. DNA TEs can also achieve dominance under conditions favoring their intragenomic spread, and their intergenomic invasiveness provides opportunities for cross-species entry. While mammalian genomes have been extensively sampled, some of the largest non-mammalian taxonomic groups to date include only one or very few sequenced representatives, and a number of phyla remain completely unexplored. Wider phylogenetic sampling of sequenced genomes is sorely needed and should include at a minimum several representatives from each major taxonomic group, such as a phylum.

The animal systems described above represent the areas to watch, for which further comparative genomic and transcriptomic studies can be expected to bring much progress in the near future. It should be emphasized that the forthcoming genome assemblies should represent high-quality drafts in order to maximize the value of sequence information. One of the major problems hampering the efforts in neopolyploid and synthetic polyploid genome sequencing is the necessity to assemble haplotypes on a large scale with a high degree of confidence. If technical challenges in haplotype assembly are to be overcome sooner rather than later, we may expect immediate advances in understanding the corresponding genome structures. Many vertebrate species of interest (fish, amphibians, reptiles) will definitely get a boost from the Genome 10K project [Genome10K Community of Scientists, 2009]. In conjunction with whole-genome sequencing, the concomitant analyses of transcriptomes and epigenomes would be of particular importance, as the generation of these datasets is relatively easy, but their proper analysis strongly depends on genome availability. Although TE activation upon 'genomic shock' is not inevitable, as it may constitute only one of many possible manifestations of the breakdown of epigenetic TE controls, it certainly occurs in many cases and has the potential to considerably change the outcomes of hybridization and polyploidization events. While we cannot yet provide a comprehensive picture of TE behavior in hybrid and polyploid animal systems, it appears to be a very promising direction for future investigations. Overall, there is little doubt that TE studies in animal species in the context of hybrid evolution and speciation, fueled by advances in genomics and epigenomics, will emerge as a distinctive new trend in polyploidy research.

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