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Genome structure of bdelloid rotifers: shaped by asexuality or desiccation?

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Abstract. Bdelloid rotifers are microscopic invertebrate animals best known for their ancient asexuality and the ability to survive desiccation at any life stage. Both factors are expected to have a profound influence on their genome structure. Recent molecular studies demonstrated that, while the gene-rich regions of bdelloid genomes are organized as co-linear pairs of closely related sequences and depleted in repetitive DNA, subtelomeric regions harbor diverse transposable elements and horizontally acquired genes of foreign origin. While asexuality is expected to result in depletion of deleterious transposons, only desiccation appears to have the power to produce all of the uncovered genomic peculiarities. Repair of desiccation-induced DNA damage would require the presence of a homologous template, maintaining co-linear pairs in gene-rich regions, and selecting against insertion of repetitive DNA which might cause chromosomal rearrangements. Desiccation may also induce a transient state of competence in recovering animals, allowing them to acquire environmental DNA. Even if bdelloids engage in rare or obscure forms of sexual reproduction, all these features could still be present. The relative contribution of asexuality and desiccation to genome organization may be clarified by analyzing whole-genome sequences and comparing foreign gene and transposon content in species which lost the ability to survive desiccation.

Introduction

Rotifers of the class Bdelloidea are microscopic invertebrate animals which populate nearly every possible freshwater habitat on Earth, including those that remain wet only for a short period of time. Discovered by Antony van Leeuwenhoek (1677) and noticed for the absence of males two hundred years later (Hudson and Gosse 1886), they continued to attract attention of amateur and professional naturalists around the world ever since (for recent review, see Mark Welch et al. 2009). More than four hundred described species of bdelloid rotifers (Segers 2007)

are classified in four families and nineteen genera within the obligatorily asexual class Bdelloidea, which represents a sister taxon to the facultatively asexual rotifers of the class Monogononta (Mark Welch 2001, Dunn et al. 2008; but see Witek et al. 2008). Bdelloid rotifers reproduce exclusively by parthenogenesis, whereby eggs are formed by two successive mitotic divisions of resting oocytes, in the absence of chromosome pairing or reduction in chromosome numbers (Hsu 1956a,b). It is thought that bdelloids have remained asexual for more than 40 million years (Mark Welch and Meselson 2000). In contrast, monogonont rotifers are facultatively asexual, reproducing by parthenogenesis when environmental conditions favor rapid population growth, and switching to sexual reproduction when their density reaches a certain threshold (Wallace et al. 2006). Bdelloids are also known for their extreme tolerance to desiccation at the adult stage of their life cycle, being able to sustain almost complete loss of bodily fluids in the state of anhydrobiosis for a prolonged period of time, and then come back to life once water is back (Ricci 1998).

Exploration of the genomic landscape in bdelloid rotifers has begun relatively recently, stimulated by the expectations that genome survey studies should yield insights into molecular signatures of asexuality, and reveal, at least in part, footprints of DNA repair events associated with double-strand breakage following recovery from anhydrobiosis. Below, we summarize and discuss recent information on the molecular structure of bdelloid genomes that reveals a number of unique features, possibly conferred by their long-term asexuality and/or the unusual lifestyle involving repeated cycles of desiccation and rehydration. We also present our recent data which are relevant to the topic of this review, and discuss how these findings add to the overall picture of bdelloid genome organization and evolution.

Methods

Cloning LEA genes from Adineta vaga

Fragments of *Ar-lea-1* genes were amplified from genomic DNA of *Adineta ricciae* with primers GAAACTTCCCCGAAAAATGAAC / CCTCGCTTAACACCATCTTTTG (Ar-lea-1A) and AGAAATCAGCCACAGAACAAGC / TCGTAGTTTTTCGCCTCGTTTA (Ar-lea-1B) and used to probe the *A. vaga* genomic library (Hur et al. 2009). Three fosmid clones representing distinct copies of *Av-lea-1* genes were sequenced, assembled and annotated as described previously (Gladyshev and Arkhipova 2007). Sequences obtained in this study were deposited in GenBank under accession numbers GU373045-GU373047.

Monitoring DNA repair after irradiation

An exponentially growing culture of *A. vaga* was starved for 24 hours before being harvested. Animals were chilled to 4^{o}C, dislodged from the bottom of a 15-cm plastic petri dish with a gentle jet of chilled culture medium, allowed to sediment, centrifuged briefly at 500g, and resuspended in 0.7 ml of fresh chilled culture medium. 100-ul aliquots of the resuspended rotifer culture were pipetted into 1.7-ml centrifuge tubes, and the tubes were inverted and irradiated at room temperature for 5 hours at 120 Gy/hour (Gladyshev and Meselson 2008). Tubes were removed from the irradiator and allowed to sit at room temperature for defined time intervals before being placed on ice. Once all tubes were on ice, rotifers were centrifuged briefly at 10000g, and following the removal of 70 ul of clear supernatant, animals were warmed up to 40^{o}C, mixed with 40 ul of 1% melted low-melting point agarose, poured into molds, digested with proteinase K in agarose plugs, and genomic DNA was resolved by pulse-field gel electrophoresis and visualized as described previously (Gladyshev and Meselson 2008).

Establishing heavily irradiated strains of A. vaga

A clonal isolated culture of *A. vaga* was repeatedly treated with 700 Gy of gamma radiation and allowed to recover and start reproducing between succeeding treatments. Overall, 7000 Gy were delivered to the rotifer culture over the course of 4 months, corresponding to 1 DSB per 30-35 kbp of genomic DNA. After the last treatment was administered, the culture was allowed to recover and individual animals were transferred into wells of the 96-well plate and allowed to reproduce. A new culture was started from a single F1 animal that corresponded to a single oocyte nucleus of the irradiated individual.

RESULTS AND DISCUSSION

Genome Organization

The first glimpse into the degenerately tetraploid structure of bdelloid genomes was provided by comparative analysis of 45-70 kb chromosomal regions surrounding four copies of the *hsp82* heat shock protein gene in the bdelloid *Philodina roseola* (Mark Welch et al. 2008). While gene content, order, and orientation were preserved *within* each pair of closely related co-linear sequences, only a few genes were shared *between* the two pairs of homeologous sequences that comprised the tetraploid quartet (Mark Welch et al. 2008). Multiple gene losses, and perhaps acquisitions, have apparently occurred in each homeologous pair independently (Fig. 1). Duplications could not easily account for the observed pattern, as each *hsp82*-containing contig was found to reside on a separate chromosome (Mark Welch et al. 2004). Gene copies from different co-linear pairs are extremely divergent (with K_s values up to 120%, Mark Welch et al. 2008), which was initially interpreted as the genetic distance between former alleles that had accumulated substitutions following the ancient loss of sex (Mark Welch and Meselson 2000), but is now understood to result from divergence between homeologs (Mark Welch et al. 2008).

Analysis of *hsp82*-containing contigs in another bdelloid species, *Adineta vaga*, as well as histone-containing contigs in both *P. roseola* and *A. vaga,* showed that degenerate tetraploidy was established prior to separation of the major bdelloid families (Hur et al. 2009).

Karyotypes of bdelloid rotifers are consistent with degenerate tetraploidy. *A. vaga* has 12 chromosomes of approximately equal size, corresponding to 3 quartets. Although *P. roseola* has 13 chromosomes, two of these chromosomes are most likely B (accessory) chromosomes, while two co-linear (homologous) chromosomes were apparently fused to produce a large isochromosome (Mark Welch and Meselson 1998; Mark Welch et al. 2004).

The average nucleotide sequence divergence *within* each co-linear pair, measured by a 1000-bp sliding window, is 3-5%, but in some cases it may be as high as 20 percent or as low as zero (Mark Welch et al. 2008). The occasional lack of any sequence divergence may be explained by gene conversion produced by recombinational DNA repair, which may have homogenized co-linear sequences over tens of thousands of base pairs (Hur et al. 2009). Nucleotide divergence values of a few percent observed within co-linear sequences in bdelloid genomes are comparable to the average nucleotide divergence between alleles in species with very large effective population sizes, such as the ascidian *Ciona savignyi* and the root-knot parasitic nematode *Meloidogyne incognita,* which are characterized by the genome-wide average heterozygosity of *ca*. 7% (Bird et al. 2009; Vinson et al. 2005; Small et al. 2007). This observation strongly suggests that bdelloid species are also characterized by large population sizes.

In the bdelloid *Adineta ricciae,* through cDNA sequencing and analysis, Pouchkina-Stantcheva *et al*. (2008) identified two LEA (Late Embryognesis Abundant) genes that shared pronounced structural and sequence similarity, both containing 9 short introns and having the

average K_s value of 13.5%, which was much lower than K_s of homeologous gene pairs in *A. vaga* and *P. roseola* (Mark Welch et al. 2008; Hur et al. 2009). Southern blot analysis revealed the presence of only two sequences, each corresponding to either one or another of the identified LEA genes (*Ar-lea-1A* and *Ar-lea-1B*) (Fig 2A). These results were consistent with fluorescent *in situ* hybridization (FISH) analysis, which identified two distinct spots in interphase nuclei of *A. ricciae*. Taken together, the data were interpreted as a case of accumulation of nucleotide divergence between presumed former alleles that were no longer subject to homogenization by recombinational mechanisms. Furthermore, because *Ar-lea-1A* and *Ar-lea-1B* appeared to have different physical and biochemical properties, it was concluded that these putatively former alleles underwent neofunctionalization. However, given that the genomes of bdelloid rotifers are degenerately tetraploid, and that the expected nucleotide divergence within each co-linear pair is 3-5%, the above results may be interpreted differently (Meselson and Mark Welch 2008).

We find that the genome of *A. vaga* also contains two closely related copies of *lea-1* (Fig 2B), designated as *Av-lea-1B* and *Av-lea-1B'*, as both copies appeared more similar to *Ar-lea-1B* than to *Ar-lea-1A*. Another *Av-lea-1* gene, structurally distinct from *Ar-lea-1A* or *Ar-lea-1B* and designated as *Av-lea-1C*, was found on a different fosmid. No co-linear partner of *Av-lea-1C* or putative homologs of *Av-lea-1A* could be identified in the *A. vaga* genomic library. *Av-lea-1B* and *Av-lea-1B'* reside on separate contigs that appear to be co-linear, and therefore may be subject to gene conversion associated with recombinational DNA repair. If the overall organization of the *A. ricciae* genome is similar to that of *A. vaga* and *P. roseola*, then the two identified copies of *Ar-lea-1* must represent either co-linear copies or relatively recently diverged paralogs, each represented by two identical or almost identical co-linear copies (Fig. 2C). The

latter hypothesis is favored by the functional divergence between Ar-lea-1A and Ar-lea-1B that would otherwise be erased by gene conversion.

Transposable Elements

Transposable elements (TEs) are segments of genomic DNA characterized by their ability to move around the genome, and frequently cause deleterious insertional mutations, deletions, and chromosomal rearrangements as a result of such mobility. They are present in virtually every eukaryote, and their numbers range anywhere from only a few to millions of copies per genome. It has long been thought that the asexual mode of reproduction can bring into play opposing forces which would act either to reduce or to increase the deleterious load of TEs in the genome. According to Hickey (1982), TEs are capable of spreading in sexually-reproducing populations by vertical transmission from an infected parent to the progeny, despite being deleterious to the host, while, conversely, in asexual populations the spread of TEs would be limited to horizontal transfer events, which are relatively rare. On the other hand, the ability of TEs to multiply intragenomically should cause accumulation of deleterious mutations under Muller's ratchet in the absence of sex (Muller 1964). Depending on the effective population size and other parameters, and in the absence of horizontal transmission, deleterious TEs in asexual populations can be expected either to increase indefinitely, thereby eventually driving the host population to extinction, or to become lost or domesticated (Arkhipova and Meselson 2005a). Loss of TEs may occur *via* stochastic excision, and their limitation and mutational decay could be facilitated by silencing mechanisms. In sexual species, an essential factor limiting TE proliferation is thought to be represented by synergistic selection against chromosomal abnormalities resulting from ectopic meiotic crossing-over between dispersed homologous repeats, a mechanism which is not

expected to operate in asexuals (Charlesworth and Langley 1989; Arkhipova and Meselson 2005a).

It was of interest to find out whether TE activity and their genomic distribution in bdelloid rotifers would be consistent with any of the above theories. Initial PCR-based screens for the most abundant TE superfamilies already revealed certain peculiarities of TE distribution in bdelloids: while DNA TEs were readily detectable, retrotransposons from the most prominent gypsy and LINE superfamilies were not, although all other screened eukaryotes contained them (Arkhipova and Meselson 2000). Since the propensity of DNA TEs for horizontal transfer is well known, it was not surprising that in subsequent studies their diversity was confirmed and expanded, including superfamilies such as mariner/Tc, hobo, piggyBac, foldback, and Helitron (Arkhipova and Meselson 2005b). At the same time, virtually no TEs could be detected during sequencing of 45-70 kb stretches of DNA from overlapping *A. vaga* and *P. roseola* fosmids containing single-copy genes such as *hsp82*, histones, or Hox (Mark Welch et al. 2008; Hur et al. 2009; J. Mark Welch, personal communication), covering more than 1.5 Mb in total. For comparison, even in model organisms that may be regarded as relatively TE-poor, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, the average density of TEs in gene-rich regions is 7.7-12.3 retrotransposons and 2.3-3.6 DNA TEs per Mb in *D. melanogaster* (Kaminker et al. 2002), and ~7 retrotransposons and ~19 DNA TEs per Mb in *C. elegans* (Duret et al. 2000).

Such an unusual lack of TEs in gene-rich regions of bdelloid genomes prompted us to initiate a project aimed at cloning and sequencing of bdelloid telomeres, as subterminal chromosomal regions would be expected to contain fewer essential genes and more “junk DNA”. Indeed, genome walks from cloned telomeres into subterminal regions revealed a considerable

variety of retroelements, including retrovirus-like retrotransposons *Juno* and *Vesta* with envelope-like genes (Gladyshev et al. 2007), as well as telomere-associated, endonuclease-deficient *Athena* retroelements which are specialized for addition to deprotected chromosome ends (Gladyshev and Arkhipova 2007). *Juno* and *Vesta* are LTR (long terminal repeat) retrotransposons, which are generally prone to horizontal transmission, either as virus-like entities through infection, or *via* transmission of the cytoplasmic double-stranded linear DNA intermediate (Kim et al. 1994; Jordan et al. 1999). *Athenas* are *Penelope*-like elements (PLEs) without the characteristic GIY-YIG endonuclease domain, which is normally required for PLE integration into internal regions of the chromosome (Arkhipova 2006), and they apparently help to extend chromosome termini in combination with conventional telomerase-mediated telomeric repeat addition (Gladyshev and Arkhipova 2007). Members of several families of *Athena* retroelements, mostly 5'-truncated, were found to form long head-to-tail interspersed arrays at chromosome ends, separated by short stretches of G-rich telomeric repeats likely added by telomerase.

Do bdelloid genomes carry TEs that are neither likely to be transferred horizontally nor can be regarded as providing any benefits to the host? Traditionally, the best candidates for such TEs are non-LTR retrotransposons, which are transmitted mostly vertically, and exhibit *cis*-preference, *i.e*. preferential propagation of the active copy without significant mobilization of defective members of the same family, which could be recognized *in trans* by the enzymatic machinery of the active element (Malik et al. 1998; Wei et al. 2001). Although non-LTR retrotransposons in bdelloids have remained elusive for a long time, they were recently uncovered in ribosomal DNA, which is located subtelomerically in bdelloids, and in additional subtelomeric regions. The rDNA-specific non-LTR retrotransposons, R9, insert site-specifically

into 28S ribosomal RNA genes, and cause an exceptionally long 126-bp target site duplication (Gladyshev and Arkhipova 2009a). Another non-LTR retrotransposon, *Hebe*, is unusual in not having any 5'-truncated copies, as is typical for most non-LTR retrotransposons, but contains numerous deletions which were apparently generated in the course of microhomology-mediated end-joining (Gladyshev and Arkhipova 2010). While the R9 retrotransposons possess strict sequence specificity for rDNA and lack the ability to spread throughout the genome, the presence of *Hebe*-like elements may pose a threat to an asexual lineage in terms of their potential capacity to insert into internal genomic locations and thereby promote deleterious chromosomal rearrangements. There remains, however, a possibility that *Hebe* may have developed targeting preference for subterminal chromatin, which could be mediated by the ORF1 product, as it exhibits preference for subtelomeric regions.

Overall, bdelloid retrotransposons can be characterized by a considerable diversity of families, by low copy number within each family, and by mostly subtelomeric localizaion. Comparison of TE content between telomeric/TE-rich and gene-rich regions on a pie chart (Fig. 3) clearly demonstrates the extreme compartmentalization of bdelloid TEs near telomeres, as well as their virtual exclusion from gene-rich regions, in more than two megabases of sequenced genomic DNA from *A. vaga* and *P. roseola*.

Horizontal Gene Transfer

Subtelomeric regions of bdelloid genomes also contain a large number of protein-coding sequences which are more similar to non-metazoan homologs than to metazoan ones, or for which no metazoan counterparts could be identified (Gladyshev et al. 2008). Several bdelloid genes of apparently bacterial origin, such as alanine racemase and D-alanyl-D-alanine ligase, are interrupted by canonical spliceosomal introns, which do not exist in bacteria. A few genes are

shared only between bdelloids and very small groups of bacteria and fungi, thus making the probability of inheriting such rare genes from a common ancestor exceedingly low. While the majority of foreign genes encode full-length proteins, some contain frameshifts or stop codons and are obviously non-functional. Although no direct evidence of a contemporary horizontal gene transfer (HGT) event in a bdelloid has been found to date, the presence of non-metazoan pseudogenes at rapidly evolving chromosome ends implies their relatively recent acquisition, as such decaying inserts would have undergone rapid loss in the absence of purifying selection.

While HGT is quite common among prokaryotes and unicellular eukaryotes, multicellular eukaryotes such as plants and animals typically rely on internal sources of genetic variability, such as gene duplication and subsequent diversification of gene expression, for successful adaptation to the new environments. Previously, HGT in metazoans was shown to result from long-term symbiotic or parasitic relationships between the non-metazoan donor and the metazoan host (reviewed in Keeling and Palmer 2008). For example, a large portion of the genome of *Wolbachia*, a common bacterial endoparasite of insects and worms, has been detected in the *Drosophila* genome, and certain *Wolbachia* genes were also found in nematodes (Hotopp et al. 2007). Yet another case of metazoan HGT has been described in the sea slug, *Elysia chlorotica*, which preys on algae but can retain photosynthesizing algal plastids within its digestive epithelium, and has captured an algal nuclear gene *psbO* required for plastid viability (Rumpho et al. 2008). Parasitic root-knot nematodes have acquired genes needed for enzymatic cell-wall degradation from bacteria, and it is thought that the origin of plant parasitism in the common ancestor of these nematodes was associated with HGT (Bird et al. 2009; Mitreva et al. 2009). The overwhelming diversity of putative sources of foreign genes in bdelloid genomes, which come from different bacterial subdivisions as well as from fungal and plant kingdoms,

strongly argues against systematic involvement of any given endosymbiont or parasite in gene transfer, although isolated events of this kind cannot of course be excluded.

Could any selective advantage be provided to bdelloid species by acquisition of genes from foreign sources? Although we do not yet know the answer to this question, it is worth noting that those acquired genes which apparently retained their function belong to the category of "operational" rather than "informational" genes (Jain et al. 1999). In other words, the retained genes may have been recruited to perform a specialized function (*e.g*. carbohydrate decomposition, such as bacterial β-D-xylosidase), while the decaying genes constituted parts of a complex metabolic pathway or process in the previous host and could not be easily plugged into the regulatory systems of the new host (*e.g*. signal transduction, such as serine/threonine kinase of plant origin).

Desiccation and radiation resistance

The majority of bdelloid species are characterized by the ability to survive desiccation without assuming a special developmental form, such as cysts and resting eggs of certain desiccation-resistant nematodes, tardigrades, brine shrimps, or monogonont rotifers (Aguilera and Karel 1997; Clegg 2001; Wallace et al. 2006). When humidity starts to decrease, bdelloid rotifers undergo physiological adjustments and contract into a compact body shape called a *tun* (Ricci et al. 2003). Desiccated animals retain very little liquid inside, and they cope with such a drastic water loss by producing protective hydrophilic molecules, *e.g.*, LEA proteins described above, which may substitute for water by forming an extended network of hydrogen bonds. Interestingly, bdelloids appear not to rely on the non-reducing sugar trehalose or other disaccharides for these purposes (Lapinski and Tunnacliffe 2003), hinting at a possibility of utilizing perhaps even more potent protector molecules.

In the radiation- and desiccation-resistant bacterium *Deinococcus radiodurans*, which can reassemble its chromosomes after exposure to several thousand grays (Gy) of gamma irradiation, the extreme radiation resistance has likely evolved as an adaptation to frequent DNA damage incurred during desiccation, and is genetically linked to exceptionally efficient DNA repair (Mattimore and Battista 1996; Zahradka et al. 2006). Remarkably, bdelloid rotifers are also highly resistant to ionizing radiation, being able to resume reproduction after receiving more than a thousand Gy of gamma irradiation from a 137-Cs source, while the decrease in reproductive capacity of irradiated monogononts is similar to that of worms and diverse insects (Gladyshev and Meselson 2008). Because the yield of double-strand breaks (DSBs) per Mb of DNA per Gy in bdelloids is similar to other organisms, it was concluded that either bdelloids possessed exceptionally efficient DNA repair machinery, unprecedented in the animal world, or that they can better protect conventional DNA repair proteins from oxidation by free radicals induced by gamma irradiation. However, a third possibility existed that, while genomic DNA in somatic nuclei was readily broken by ionizing radiation, DNA in resting oocytes was at least partially protected, and thus oocyte nuclei never incurred and repaired nearly as many double-strand DNA breaks as somatic nuclei.

As DNA repair is clearly evident in somatic nuclei (Fig. 4A), it was of interest to examine the effects of excessive DNA breakage on the TE content of bdelloid genomes, reasoning that such DNA could pose a problem for recombinational DNA repair, resulting in deletions and rearrangements that could be readily detected by Southern blot analysis. An isolated culture of *A. vaga* was started from a single egg and repeatedly irradiated over the course of four months, during which no new animals were introduced. If oocyte DNA were as prone to breakage by ionizing radiation as somatic DNA, the dose of 7000 Gy would translate

into 1 DSB per 30 kbp of genomic DNA. Preliminary analysis of mobile DNA content in irradiated strains of *A. vaga,* using a mariner-like *Avmar1* DNA transposon as a probe, demonstrated that some of the *Avmar1* copies could have been lost from the genome (Fig. 4B). Because such changes had to occur in the germ-line nuclei, these data suggest that oocyte DNA can be broken and rejoined as efficiently as somatic DNA, and that TE copies can be purged from the genome as a result of induced DNA damage.

Asexuality or desiccation?

The presence of gene-rich regions as co-linear pairs, the conspicuous absence of mobile and other repetitive DNA from these gene-rich regions, and the accumulation of TEs and foreign genes near chromosome ends constitute distinctive features of bdelloid genomes. It is expected that examination of the overall bdelloid genome structure, as well as TE content, should help to determine the relative contributions of asexuality and anhydrobiosis to the emergence of these characteristic features. Below, we will assess the potential of each factor to significantly impact genome structure.

The existence of co-linear nearly-identical gene-rich pairs of chromosomal segments requires frequent homogenization of otherwise slowly diverging sequences. Both genetic drift and gene conversion can accomplish this task. While the former mechanism strictly depends on sexual reproduction, the latter can operate in sexual as well as in asexual lineages. Individual members of co-linear pairs have the potential to participate in homologous repair of DSBs in genomic DNA (Mark Welch et al. 2008; Gladyshev and Meselson 2008).

The lack of mobile DNA in gene-rich regions may be readily explained by repeated cycles of desiccation-induced breakage and repair of bdelloid genomic DNA (Gladyshev and Meselson 2008; Dolgin and Charlesworth 2008). During recombinational DNA repair, ectopic

crossing-over between dispersed repetitive sequences may lead to deleterious chromosomal rearrangements, effectively selecting against high repeat content (Charlesworth and Langley 1989). Direct evidence of TE-mediated genome restructuring after radiation-induced DNA breakage was recently provided by Argueso et al. (2008), who observed that the majority of chromosomal aberrations in irradiated diploid yeast, *Saccharomyces cerevisiae*, result from recombination between nonallelic Ty1 retrotransposons. This study was carried out on yeast cells arrested in the G2 phase, which could conceivably use sister chromatids for recombinational DNA repair. It may be argued that, apart from the lack of mixis that otherwise enables TEs to proliferate in sexual populations, DNA breakage and repair associated with cycles of desiccation and rehydration may also limit the number of TEs in bdelloid genomes, providing a non-meiotic route for the elimination of TEs by DSB repair *via* ectopic recombination, which triggers deleterious translocations and deletions and imposes synergistic selection against TEs. Frequent rounds of DNA breakage and repair would be constantly selecting for genotypes with the lowest number of TEs, minimizing the chance of producing deleterious chromosomal rearrangements in bdelloids recovering from desiccation. The strength of negative selection will be proportional to the square of the number of TEs, sufficient to neutralize the propensity of TEs to multiply exponentially. Thus, DNA repair following desiccation would essentially act as a substitute for meiotic recombination in terms of limiting TE load.

Barring sexual reproduction, the presence of parasitic TEs in an anciently asexual population implies that they enter by horizontal transmission, while being kept at low copy number by mechanisms of silencing and selection. On this view, the presence of a non-LTR retrotransposon such as *Hebe,* which is expected to be vertically-transmitted, may be interpreted as evidence for sexual exchange in bdelloid populations. The Darwinulid ostracods, thought to

be anciently asexual, were also reported to contain non-LTR retrotransposons (Schön and Arkhipova 2006), and living males were found in a Japanese population (Smith et al. 2006; but see Schön et al. 2009). However, bdelloid telomeric regions do contain a substantial number of TEs which entered the genome horizontally (see above). One of the most curious cases of such transmission is an IS5-like transposon of apparently bacterial origin, which was found inserted into a eukaryotic transposon at one of the *A. vaga* telomeres (Gladyshev and Arkhipova 2009b). This TE, however, probably failed to adapt to the new host and never became capable of proliferating within the bdelloid genome, being transcriptionally inactive and present only as a single copy. It is intriguing that foreign genes, both prokaryotic and eukaryotic in origin, have apparently been more successful in adapting to the bdelloid genomic environment than certain TEs.

Frequent cycles of desiccation and rehydration may also be responsible for the observed massive horizontal gene transfer into subtelomeric regions of bdelloid genome. The dominance of vertical inheritance in Metazoa rests on anatomical barriers between germ-line nuclei and somatic tissues that may often come in contact with exogenous DNA. If the barrier becomes compromised, one should not be surprised to find substantial levels of HGT even in Metazoa. As bdelloid oocyte nuclei are already formed at the moment of hatching and reside in the immediate vicinity of the gut, they may become a target for ingested foreign DNA, should the integrity of the gut lining become compromised. Bdelloid rotifers do not discriminate between food particles, and at any given time their guts may contain semi-digested DNA of diverse phylogenetic origin. The phylogenetic spectrum of ingested DNA may be even wider than their immediate food sources, since in aquatic and soil environments free DNA can be absorbed on mineral surfaces or bound by complex organic molecules (Vlassov et al. 2007), and may easily find its way into the bdelloid gut. A single desiccation event may suffice to induce transient damage of the gut and the

ovary, facilitating the passage of foreign DNA. Once the exogenous DNA ends up in the vicinity of resting oocyte nuclei, it may be integrated into the germ-line DNA, either homologously or ectopically. If conspecific DNA is absorbed together with other exogenous DNA, it could potentially replace endogenous sequences by homologous recombination. Such rare events may provide the physical foundation of genetic exchange among conspecific individuals in the absence of sexual reproduction. However, so far only ectopic integrations of foreign DNA at subtelomeric locations have been detected. It is possible that foreign DNA may be occasionally attached directly to deprotected telomeres, perhaps in the form of a mini-end-to-end fusion event if a short stretch of telomeric repeats is also attached to the incoming linear DNA fragment. Indeed, we observed a few cases in which the boundary between bdelloid and foreign DNA contained short stretches of telomeric repeats joined together in inverted orientation (I.A. and E.G., unpublished observations).

Would the soon-to-be determined whole-genome sequence of a bdelloid rotifer allow us to unambiguously discriminate whether bdelloid asexuality or their unusual lifestyle contributed most to their peculiar genome organization? The answer to this question may lie in comparative molecular analyses of genomes of bdelloid species which are asexual, but have lost the ability to withstand desiccation, as they underwent secondary adaptation to non-ephemeral aquatic environments (Ricci 1998). In addition, comparison of distribution of full-length and truncated TE insertions on a genome-wide scale should help to determine whether longer insertions are under-represented in gene-rich regions, as may be expected if ectopic recombination is the major determinant of negative selection acting on TEs (Song and Boissinot 2007). We will undoubtedly know a lot more about bdelloid TE content and distribution when the first whole-genome sequence of a bdelloid, *Adineta vaga*, is completed during the coming year, and will probably

understand even more when the genomes of monogonont rotifers also become available for comparison.

Concluding Remarks

During the past few years, considerable progress has been made towards understanding the basic genome organization in rotifers of Class Bdelloidea. Even the relatively limited initial genome survey studies, which included selected chromosomal regions comprising a little more than one percent of total genomic DNA, revealed certain peculiar features of bdelloid genome structure, such as degenerate tetraploidy, extreme compartmentalization of mobile elements, and presence of large amounts of foreign DNA in subtelomeric regions. Two major factors may have contributed to shaping bdelloid genomes - ancient loss of sex and the unusual lifestyle which involves repeated cycles of desiccation and rehydration. While asexuality may account for some of the observed features, only desiccation appears to have the power to produce all of the uncovered genomic peculiarities. It may be hoped that larger amounts of sequence information from the bdelloid genome project, as well as comparative studies of rotifers with different lifestyles, will eventually help to elucidate the major driving forces in bdelloid genome evolution.

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Figure Legends

Fig. 1. Schematic representation of two homeologous lineages, A and B, which were formed as a result of whole-genome duplication and subsequent differential gene loss. Only three genes, ***a***, ***d***, and ***g***, which are connected by solid lines, remain common to both lineages. For simplicity, the diagram depicts only gene deletions, although additions are also possible. Adapted from Mark Welch et al. (2008) and Hur et al. (2009).

Fig. 2. LEA genes in *Adineta vaga* and *Adineta ricciae*.

(**A**) The proposed genomic configuration of LEA genes in *A. ricciae*, and predicted results of Southern blot analysis using the 3' probe (black rectangle; see text) and digestion with two restriction enzymes (*Dra*I and *Eco*RI, see text). Ar-lea-1A and Ar-lea-1B represent former alleles that underwent neofunctionalization in *A. ricciae* (Pouchkina-Stantcheva et al., 2007).

(**B**) The observed genomic structure of LEA genes in *A. vaga*. Two closely related Av-lea-1B genes (B and B') are co-linear and reside in close proximity to a tandem duplication (not shown). A similar duplication might have created Ar-lea-1A and Ar-lea-1B genes. Another lea-1 gene, Av-lea-1C, is found on a separate subtelomeric genomic contig. No Av-lea-1A homologs were found.

(**C**) The alternative genomic configuration of LEA genes in *A. ricciae* is also consistent with sequencing, Southern blot and FISH results. Ar-lea-1A and Ar-lea-1B are paralogous genes produced by tandem duplication, each represented by two co-linear copies (Ar-lea-1A and A' and Ar-lea1-B and B', respectively) which underwent recent homogenization, possibly by gene conversion.

Fig. 3. Comparison between telomeric/TE-rich (**A**) and gene-rich (**B**) regions of bdelloid genomes, with *ca*. 1 Mb of genomic DNA included in each dataset. Adapted from Gladyshev et al. (2008). The charts illustrate the relative abundance of foreign genes, metazoan genes, "intermediate" (which could be assigned to either foreign or metazoan) genes, hypothetical ORFs with no similarity to known genes, and various TEs (DNA transposons, LTR retrotransposons, and telomere-specific retroelements) in the two datasets.

Fig. 4. Double-strand break repair in bdelloid rotifers after exposure to ionizing radiation.
(**A**) *A. vaga* genomic DNA is efficiently repaired after irradiation. Equal numbers of animals were distributed into 1.7-ml tubes, irradiated with 700 Gy from a 137-Cs source, allowed to recover for a given period of time, and placed on ice. C, unirradiated control; M, molecular weight markers (yeast chromosomes).
(**B**) A clonal population of *A. vaga* (R10) was obtained by irradiating an isolated culture of *A. vaga* with 7000 Gy over four months. Loss and rearrangement of *Avmar1* TE DNA is suggested by Southern blot analysis. Asterisks indicate band losses, rectangle – band gain or alternative rearrangement.

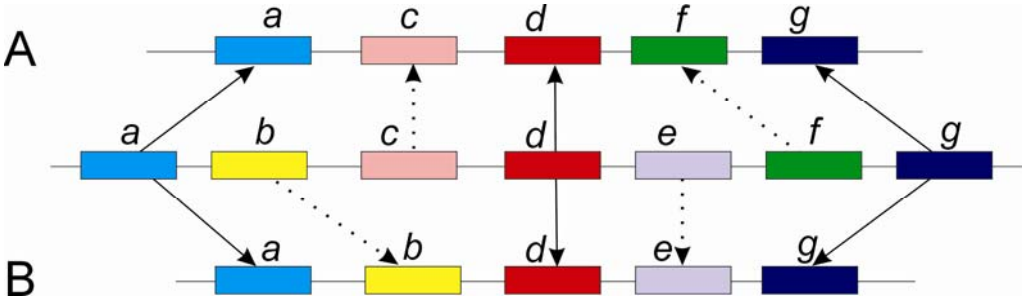
A
a
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Fig.1

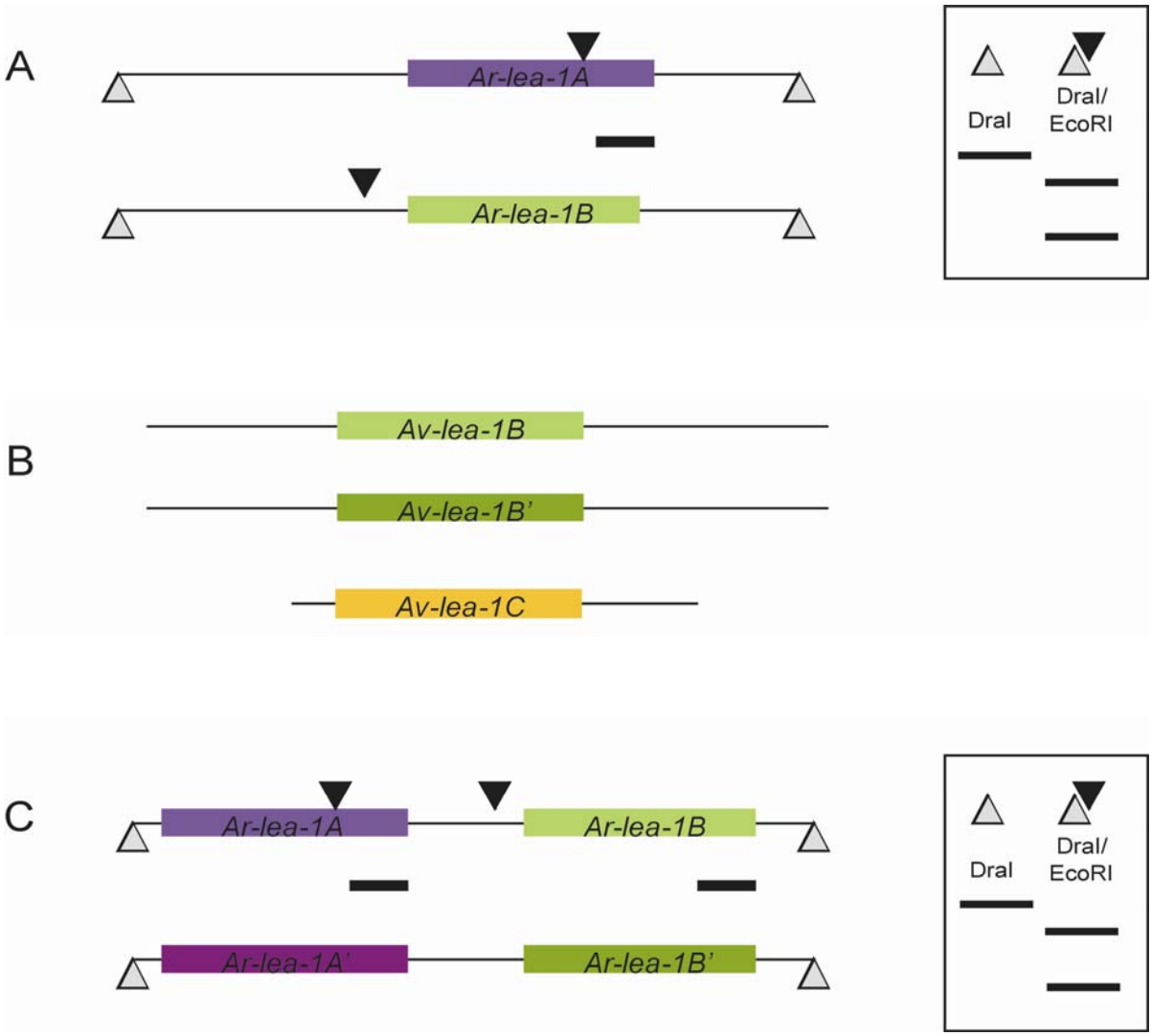
A
Ar-lea-1A
Ar-lea-1B
DraI
DraI/
EcoRI
B
Av-lea-1B
Av-lea-1B'
Av-lea-1C
C
Ar-lea-1A
Ar-lea-1B
Ar-lea-1A'
Ar-lea-1B'
DraI
DraI/
EcoRI

Fig.2

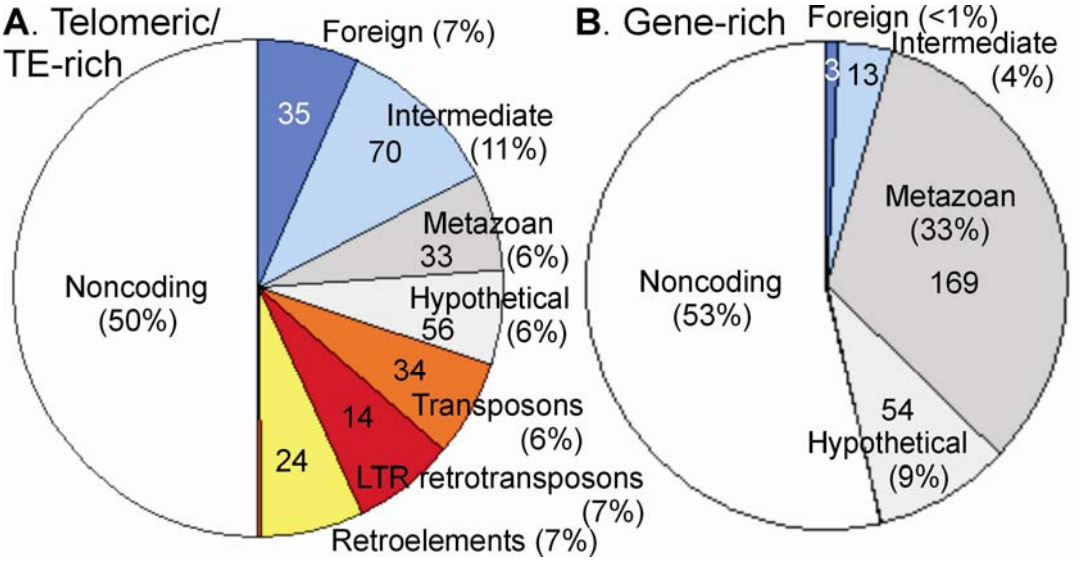
A. Telomeric/
TE-rich
Foreign (7%)
35
Intermediate
70
(11%)
Metazoan
33
(6%)
Hypothetical
56
(6%)
34
Transposons
(6%)
14
LTR retrotransposons
(7%)
24
Retroelements (7%)
Noncoding
(50%)
B. Gene-rich
Foreign (<1%)
3
13
Intermediate
(4%)
Metazoan
(33%)
169
Noncoding
(53%)
54
Hypothetical
(9%)

Fig. 3

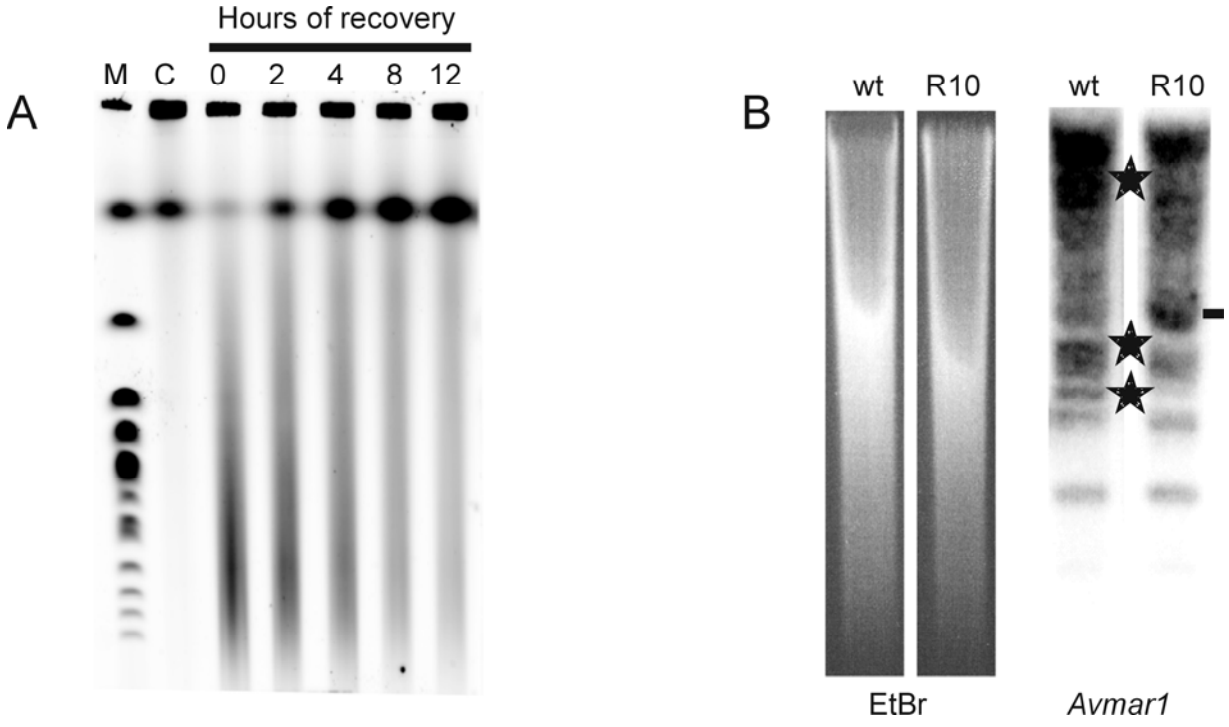
A
Hours of recovery
M
C
0
2
4
8
12
B
wt
R10
wt
R10
EtBr
Avmar1

Fig. 4