Trends in Genetics

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Review

Recombination in bdelloid rotifer genomes: asexuality, transfer and stress

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Bdelloid rotifers constitute a class of microscopic animals living in freshwater habitats worldwide. Several strange features of bdelloids have drawn attention: their ability to tolerate desiccation and other stresses, a lack of reported males across the clade despite centuries of study, and unusually high numbers of horizontally acquired, non-metazoan genes. Genome sequencing is transforming our understanding of their lifestyle and its consequences, while in turn providing wider insights about recombination and genome organisation in animals. Many questions remain, not least how to reconcile apparent genomic signatures of sex with the continued absence of reported males, why bdelloids have so many horizontally acquired genes, and how their remarkable ability to survive stress interacts with recombination and other genomic processes.

Highlights

Bdelloid rotifer genomes show signatures of asyngamous recombination leading to loss of heterozygosity. This has been hypothesised to occur via chromosome pairing during egg formation through a meiosis-like but non-reductional process.

Evidence of genetic exchange in bdelloid populations requires further validation in our view.

Around 10% of bdelloid genes have been acquired horizontally from other kingdoms of life; the mechanisms and consequences of high horizontal gene transfer (HGT) prevalence remain to be confirmed by functional and comparative studies.

Bdelloids tolerate desiccation and radiation stress, and appear to repair the resulting DNA double-strand breaks by different mechanisms in the soma versus germline; other aspects of stress tolerance remain to be investigated.

Links between parthenogenesis, desiccation tolerance and horizontal gene transfer have been hypothesised, but several key steps remain to be tested.

What are bdelloid rotifers and why study their genomes?

Early genome sequencing focused on a small set of model organisms, chosen to represent major branches of the tree of life, in an initial search for general rules that govern genome organisation and evolution. As genomes are sequenced from an expanding array of taxa [1], it has become clear that these rules can vary strikingly among organisms. Consequently, diverse taxa from distinct phylogenetic clades might provide interesting cases for investigating particular genomic processes. The limiting step now is often not sequencing a genome, but knowing enough about the life history, ecology, and cellular biology of the organism to interpret genomic patterns.

Bdelloid rotifers have long attracted interest as a case that seems to push the limits of metazoan biology, initially in terms of ecology and reproduction, and more recently with respect to genomics. These microscopic invertebrates, typically <0.5 mm long, are common worldwide in freshwater and especially ephemeral habitats such as the water film on mosses and lichens [2], where even a patch the size of a coin may harbour hundreds of individuals (Figure 1A). Discovered by Antonie van Leeuwenhoek in the 1670s [3,4], over 460 species are now known, comprising a monophyletic clade within the phylum Rotifera (and superphylum Gnathifera [5]) with an estimated crown age of at least 50 million years [6,7]. By 1886, Hudson and Gosse noted a 'strange fact' about bdelloids: no males had been seen, despite 'a century and half of observation' [8]. Another century and half later, this strange fact remains [9,10], despite direct observation of hundreds of thousands of individuals [11] and the discovery of males in other putative '**ancient asexual**' (see Glossary) groups [12–14]. As the largest clade of animals that lack known males, they have sometimes been called an '**evolutionary scandal**' [15–17] because they seem to challenge theories for the adaptive benefits of sexual reproduction [18].

Aside from the strange lack of males, bdelloid rotifers have drawn interest for their extraordinary stress tolerance, especially in relation to desiccation and radiation [19–22]. After adding water to some dry dirt collected from a gutter, van Leeuwenhoek observed 'animalcules' rolled-up into ovals which, within 30 min, stretched out their bodies and started swimming around [23].

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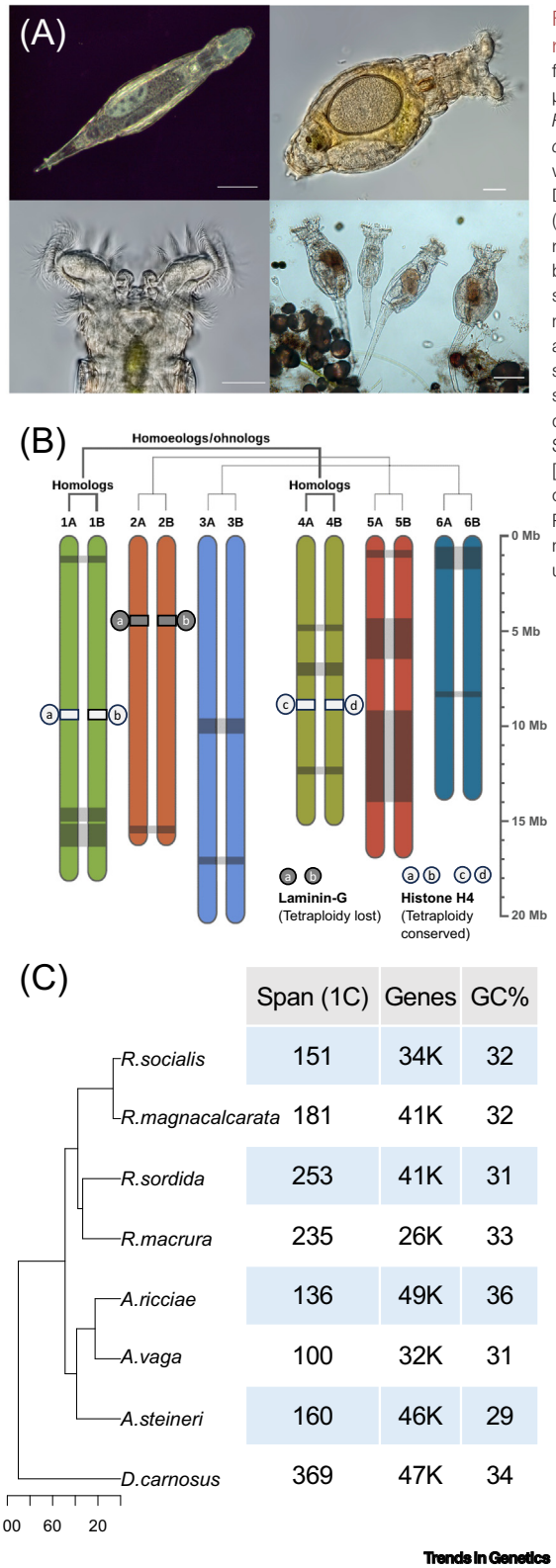

Figure 1. (A) Micrographs of some bdelloid rotifers used in genomic studies. Clockwise from top left (scale bar length): *Adineta vaga* (30 μm), *Macrotrachela quadricornifera* (25 μm), *Rotaria macruca* (50 μm), and *Didymodactylos carnosus* (25 μm). *A. vaga* by C. Wilson, others with permission by M. Plewka (plingfactory.de). (B) Degenerate tetraploidy of the *A. vaga* genome (modified from [36]). Shading indicates regions of loss of heterozygosity (LOH) between diploid chromosomes. Other bdelloid species may have different chromosome numbers. Examples of genes retaining all four ancestral copies versus just two copies are shown. (C) Phylogeny and comparative genomic statistics for species of bdelloid rotifers with crude date calibration in millions of years (based on [7]). Span indicates the haploid assembly size [34,36,71], number of predicted protein-coding genes (×1000), and GC content. References discuss uncertainties in span and gene number owing to fragmented assemblies and uneven haplotype collapse in most species.

Glossary

Allele sharing: a class of allele relationships postulated as incompatible with obligate asexuality: for example, a tree topology where alleles of an individual cluster with alleles of two other individuals.

Ancient asexual: groups proposed to have lacked sexual reproduction for extended evolutionary periods, typically exceeding 100 000 years. Estimates of lineage age and evidence of truly obligate asexuality are subject to debate and uncertainty.

Anhydrobiosis: the phenomenon by which an organism can survive desiccation. In bdelloids, this can occur at any life stage, with adults contracting into dormant 'tuns' that revive and resume reproduction on rehydration.

Asyngamous recombination: recombination occurring within a parthenogenetic line of descent, without the fusion of gametes (syngamy). This could include gene conversion or crossing over, either mitotic or associated with an abortive meiosis.

Degenerate tetraploidy: the signal of ancient genome duplication or hybridisation, such that genes group into homologous pairs, which then further pair into divergent ohnologs (duplication scenario) or homoeologs (hybridisation scenario) (Figure 1). The scenario in bdelloids is possibly unknowable given the long time since this unique event.

DNA double-stranded breaks (DSBs): both DNA strands are broken in close enough proximity that the double helix is cut in two. Caused by ROS resulting from desiccation, IR exposure, and other stressors, or by enzymes during meiosis and other cellular processes.

Evolutionary scandal: from a quote about bdelloid rotifers by Maynard Smith (1986): a 'scandal' because these rotifers seem to contradict evolutionary theories predicting extinction for obligate asexuals.

Genetic exchange: any process that brings together genetic material from different individuals, leading to 'reshuffling' of genotypes. It encompasses sexual reproduction in the form of outcrossing and other mechanisms (e.g., parasexuality or near HGT).

Genetic system: the way that genetic information is organised and transmitted between generations.

HGTc: HGT candidate gene, defined as a gene showing closer protein sequence similarity to non-metazoan sequences

These ovals, known as 'tuns' or sometimes 'xerosomes', were bdelloid rotifers in the state of **anhydrobiosis**, a form of dormancy they undergo frequently and repeatedly in nature for weeks at a time, apparently without severe fitness consequences [24]. In both the desiccated and hydrated state, bdelloids can tolerate massive doses of ionising radiation (IR), making them of potential interest for space research [21,25]. Desiccation and radiation both result in cellular and DNA damage which bdelloids can resist or repair to an unusual degree [26–28]. With respect to radiation and probably other stressors, the extreme resilience of bdelloid rotifers matches or exceeds that of tardigrades [19], although their media profile has yet to catch up.

Together, these features sparked interest in genomic signatures and mechanisms that might reflect the unusual lifestyles of bdelloids. DNA sequencing began in 2000 with attempts to test early genetic predictions of ancient asexuality [29,30]. The first transcriptomes and a genome assembly arrived a decade later [31–33], followed by comparative genomics [34,35], a chromosome-scale assembly (Figure 1B) [36], and population genomic data by the 2020s [35,37,38]. Bdelloid genome assemblies range from 135 to 369 Mb in estimated haploid span and contain between 25 000 and 50 000 predicted protein-coding genes [34,35] (Figure 1C). All bdelloid genomes so far sequenced display a signature of **degenerate tetraploidy**: while genes group into homologous pairs with relatively low divergence (0.03–4.5%) (Figure 1B), many but not all homologs group further into pairs of distantly related copies called homoeologs (or ohnologs) that typically show >30% sequence divergence at the protein level, consistent with an ancient hybridisation (or duplication) event. The chromosomal assembly of *Adineta vaga* has six diploid pairs of homologous and colinear chromosomes that also group into three homoeologous sets. Over 30% of coding genes are present in four copies across two pairs of homoeologous chromosomes [36] (Figure 1B), while other genes have lost one homeologous pair.

Genomic investigations revealed yet another 'strange fact' about bdelloids. Over 8% of their genes appear to have been acquired via **horizontal gene transfer (HGT)** from non-metazoans, including bacteria, fungi, and plants [31,34,36,39,40]. This level (Figure 2A) is much higher than that reported in other animals [41], and includes genes with biochemical functions that animals normally lack [32,42].

The combination of (i) potential long-term asexuality, (ii) stress tolerance (especially DNA repair capabilities), and (iii) high levels of HGT makes bdelloids a fascinating system for investigating how and why DNA is recombined in animals. How does their natural history relate to their **genetic system** and its functional and evolutionary consequences? Do they possess genetic mechanisms that other animals lack? This review discusses key research questions and opportunities in bdelloid genomics. We outline the latest findings and how they relate to the aforementioned three strange facts. Given the challenges in connecting genomic patterns in bdelloids to their presumed lifestyle (Box 1), we evaluate mechanisms and scenarios without making prior assumptions about the bdelloid genetic system. Recent work has brought substantial advances, but major questions remain to be resolved. The contributions of bdelloid rotifers to understanding **recombination** and DNA repair now extend well beyond their original 'claim to fame' as ancient asexuals, although that remains an important topic of active research. Work on this unusual group is indeed shedding light on the 'inconceivable possibilities' [23] of life as an animal.

How can we reconcile the absence of males with genomic signatures of sex?

Early research on bdelloid genomes sought to test classical predictions of long-term obligate asexuality [17,43]: for instance, that former diploid alleles inherited from a sexual common ancestor should diverge over time in the absence of segregation and recombination (Box 2). Later work looked for signatures of **genetic exchange** within populations, such as **incongruence** in

than to any metazoan sequences, thus inferred to be horizontally transferred. The 'candidate' flag acknowledges that alternative explanations may exist (e.g., differential loss or convergent evolution).

Horizontal gene transfer (HGT): the acquisition of genetic material via processes other than vertical descent from parent to offspring that results in reticulate evolution. 'Distant' HGT describes transfers between distantly related donor and recipient lineages (e.g., from a bacterium to an animal). 'Near' HGT describes transfers between more closely related lineages (e.g., within populations).

Incongruence: differences in branching order (topology) between loci or different copies at a single locus, which is unexpected in strictly clonal populations, but might occur by asyngamous recombination or genetic exchange.

Outcrossing: sexual reproduction involving the mating of unrelated individuals.

Recombination: any process that forms a new DNA molecule with sequences from previously different DNA molecules. Also used in an evolutionary context for the generation of new allele combinations within populations, as can arise through outcrossing or gene transfer. We use the molecular definition to distinguish between asyngamous processes and sexual reproduction.

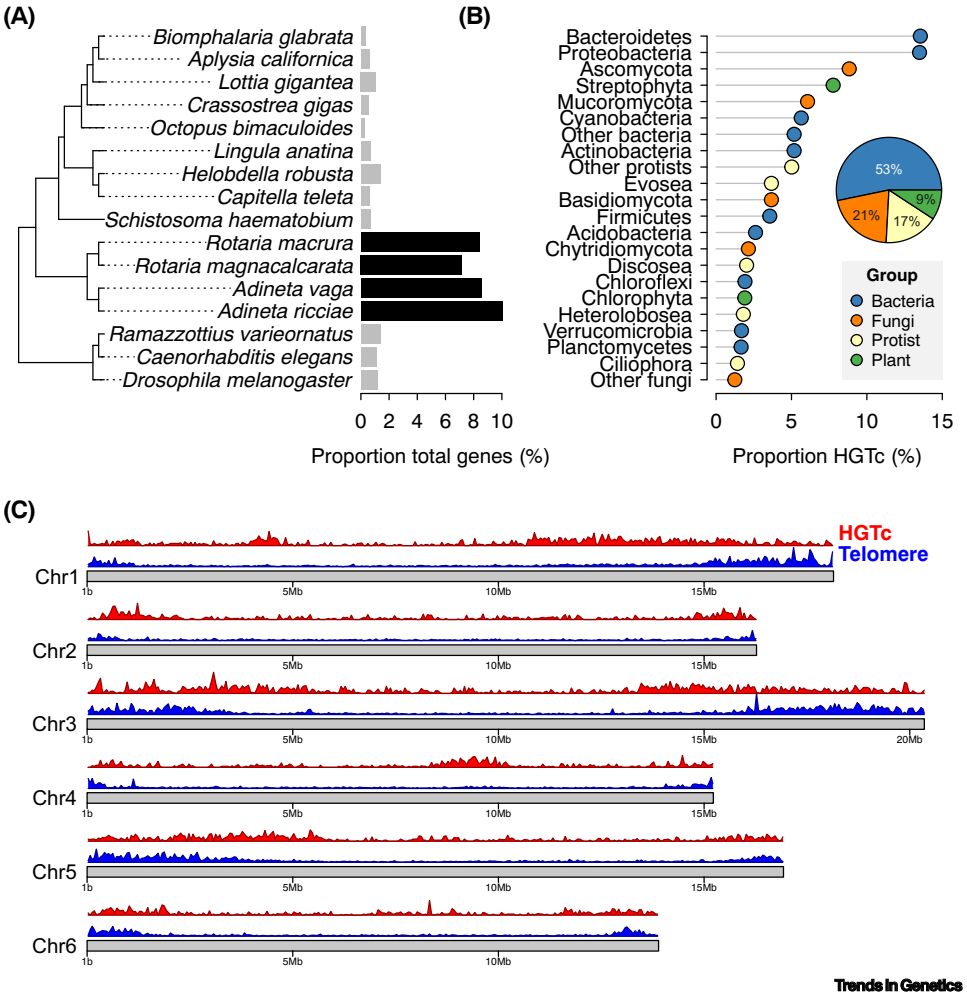

Figure 2. Horizontal gene transfer in bdelloids. (A) The percentage of genes identified as horizontally acquired from non-metazoa for a suite of animal taxa. Values for bdelloid species *Adineta vaga*, *A. ricciae*, *Rotaria macrura*, and *R. magnacalcarata* are shown with black bars. All values are taken from [34] except those for *A. vaga* [36]. (B) The proportion of horizontal gene transfer candidate (HGTc) genes attributed to different donor taxa, based on best Basic Local Alignment Search Tool match. Bacteria (blue) are most frequent, followed by fungi (orange) and plants (green). (C) The density of HGTcs (shown in red) and telomeric repeats (TGTGGG, blue) in 50 kb sliding windows along the six chromosomes of the *A. vaga* chromosomal scale haploid assembly.

genealogical relationships among loci. Despite considerable effort, however, the bdelloid genetic system has been surprisingly difficult to pin down, and several signatures reported as positive evidence either for obligate asexuality or for genetic exchange were later reinterpreted (Box 1).

At present, many features of bdelloid genomes do not seem obvious outliers when compared with sexual groups. With homologous chromosome pairs [36,44], most meiosis genes present [31,34], a diverse landscape of transposable elements (TEs) [35,45–47], heterozygosity, and other population genetic parameters within the range of sexual species [34,37], so far there is no positive genomic evidence of bdelloid ancient asexuality. Yet, these features do not constitute clear evidence for the presence of sex either. In particular, recombination between homologs within an individual has long been documented via gene conversion or larger-scale mechanisms [48,49], which must be accounted for in predictions and tests. For instance, this can explain the

Box 1. Genomic patterns in bdelloid rotifers have been reinterpreted over time

The challenges of applying older sequencing and assembly techniques to tiny non-model animals mean that several early results have later been overturned. Nuclear protein-coding sequences [30] initially seemed to show that bdelloid genera shared two deeply divergent lineages of each gene, interpreted as pairs of former alleles that had ceased to segregate and recombine millions of years ago when sex was lost in a common ancestor. Later, these divergent copies were found to result from an ancient genome duplication or hybridisation [48], with each present in paired, locally collinear, functionally diploid copies (Figure 1B in main text). In 2013, the pioneering first bdelloid genome (for *A. vaga)* appeared to show chromosomal rearrangements incompatible with meiotic pairing [31], but later long-read and *in situ* hybridisation data revealed these as assembly artefacts and resolved the same genome as six pairs of homologous chromosomes [36]. Bdelloids were initially reported to lack vertically transmitted long interspersed nuclear elements (LINE)-like and *gypsy-like* TEs, as predicted by some models of asexual evolution [29], but such elements were uncovered in later surveys [35], while evidence from other taxa has questioned the link between TEs and reproductive mode (e.g., [1,81]).

Evidence for mechanisms of genetic exchange within or between species has been similarly inconsistent. Three *Macrotrachela* lineages from distant locations seemed to show linked inheritance at multiple loci in a strikingly symmetrical pattern of allele sharing [82], interpreted as evidence of recent sexual ancestry involving non-canonical meiosis. However, genomic reanalysis of the same isolates [38] clarified that close relationships are limited to two lineages, whereas the third is closely related at only a small number of loci, four of which were initially sampled by chance. Finally, a report of inter- and intraspecific HGTs between individuals in the genus *Adineta* [83] was found to be explainable by accidental cross-contamination during sample processing [63]. In many cases, clues to the eventual reinterpretation were present in the original work as inconsistencies that required supplementary explanations beyond the main predictions, and whose importance only became clear with hindsight.

lack of deep divergence between homologous alleles without requiring the shuffling of chromosomes through fusion of gametes (syngamy) during sexual **outcrossing** (Box 2).

Progress in cytology has helped clarify how such **asyngamous recombination**, defined as recombination occurring in a parthenogenetic lineage, could shape bdelloid genomes. Although the whole class was long regarded as apomictic [10,50], recent work suggests that parthenogenesis in *A. vaga* involves a form of abortive meiosis [27,36]. This includes a non-reductional meiosis I with homologous chromosome pairing [27]. In theory, pairing could provide the opportunity for recombination between homologous chromosomes at multiple scales [36]. This could lead to loss of heterozygosity and decay of linkage disequilibrium over long genome tracts, as observed in laboratory resequencing and population samples [36,37], even when occurring in a single germline inherited parthenogenetically from mother to daughter. Evidence is now emerging to assess experimentally the mechanisms and consequences of interactions between homologous chromosomes [51].

Some recently reported genetic signatures in wild populations of *Adineta* and the distantly related species *Macrotrachela quadricornifera* might not be explained easily by asyngamous recombination alone. These include **allele sharing** trees (Box 2) and other patterns of incongruence inferred from phased sequence blocks [37,38], leading to explanations involving facultative sex and hybridisation in the recent history of these lineages. How can we reconcile these findings with the continued absence of males? We explore three possible resolutions of this apparent paradox.

There is cryptic sex but males or hermaphrodites have gone undetected

It remains possible that bdelloid rotifers do produce males that are either exceedingly rare, produced only under specific conditions, or have been taxonomically mis-assigned. Alternatively, male function in a putative bdelloid hermaphrodite could be highly reduced, anatomically unusual, or expressed only transiently. By contrast with many taxa, where outcrossing is inferred from genomic patterns but not observed directly (e.g., placozoa [52] or many protists [53]), bdelloids are cosmopolitan and easy to access, as evidenced by centuries of close observation and taxonomic descriptions of wild-caught specimens from a wide range of habitats [9,54]. Monogonont rotifers are facultative parthenogens that live in habitats overlapping those of bdelloids. Their

Box 2. What constitutes a robust genomic signature of genetic exchange?

DNA sequences record their own histories, and different histories of recombination and genetic exchange ought to leave distinct signatures [82,84]. Simple predictions regarding allele tree topologies have been used to test for asexual evolution in bdelloid rotifers (Figure I).

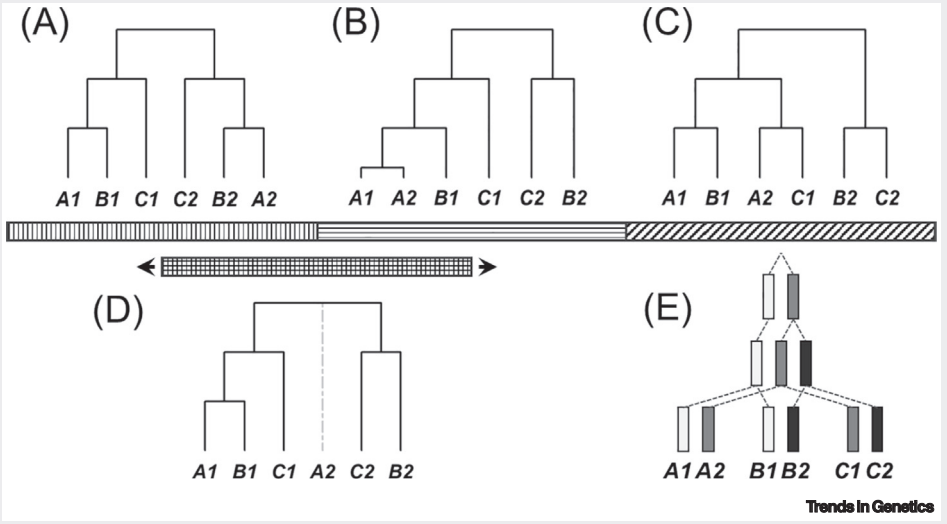

Figure I. Examples of allele tree topologies under different scenarios as explained in the main box text.
(A) If bdelloids arose from a diploid sexual ancestor but have since reproduced without any forms of recombination or genetic exchange, then homologous regions should show independent divergence over time (the 'Meselson effect') [30,43,84]. The tree for a locus (vertically hatched box) would consist of two sub-trees (for copies *1* and *2*), each recapitulating the same genealogy of individuals *A*, *B*, and *C*. With strict linkage, allele tree topologies should be the same (i.e., congruent) across all loci.
(B) If bdelloids reproduce asexually but with mechanisms for asyngamous recombination, then genealogies are not necessarily congruent across loci. For example, a gene conversion event in individual *A* leaves alleles *A1* and *A2* grouping together and an apparent sister relationship between alleles *B2* and *C2* (though with some divergence).
(C) Not all patterns of incongruence can be explained easily by asyngamous recombination alone. A class of patterns referred to as 'allele sharing' are expected with mechanisms for genetic exchange in the population. A simple case at a single locus is when alleles from one individual are reciprocal closest relatives of alleles from two other individuals [37]: for example, in a cyclical pattern, as shown (i.e., for individual *A*, allele *1* is shared with individual *B*, but allele *2* is shared with individual *C*; similarly for individuals *B* and *C*). The term is used more broadly to refer to any case where individuals share highly similar alleles at one copy, but more divergent alleles at the other diploid copy [38].
For example, the finding of incongruent allele topologies in a sample of eight *Adineta* individuals, including >12% of trees with at least one case of reciprocal allele sharing (as inferred in stringently filtered phased blocks) has been interpreted as evidence of genetic exchange [37]. Similarly, two *Macrotrachela quadricornifera* individuals shared identity across 315 out of 622 surveyed genome regions (mean length ~20 kb), whereas the remaining regions were more divergent, which was attributed to a very recent bout of sex [38].
Questions remain, however, regarding the effects of inference methods and other processes. First, asyngamous recombination can generate complex patterns of topological incongruence if trees are reconstructed from genomic windows containing a mixture of recombination histories. For instance, a gene conversion event in part of a window used for tree inference can 'pull' one copy towards an intermediate position between the two sub-trees (e.g., a region that spanned trees shown in (A) and (B) could generate the tree shown in (D), with allele *A2* in an intermediate position shown as the grey dashed branch). This could give the appearance of divergent alleles for individuals that have shared alleles at other copies/loci.
(E) Finally, other asexual processes might occur to enable a wider range of possible tree topologies. For instance, a recent autopolyploidisation event followed by independent reversals to diploidy through loss of different haplomes could generate genome-wide patterns of allele sharing.

males are reduced in size but bear a morphological similarity to females of their species, and they have been comprehensively described over the same period [8,54,55]. The single account of a possible sighting of a bdelloid male was in a mixed wild sample also containing monogonont rotifers, and framed very cautiously ('With great hesitation I venture to remark…'): two animals

resembling monogonont males were seen swimming among a dense population of bdelloids [54], but were not isolated or drawn, leaving open the possibility that they were, in fact, monogonont males. This contrasts with other putatively ancient asexual animals, where ongoing production of males has been documented for some time [12–14], and genomic evidence of their involvement in cryptic sex has recently emerged [56,57]. While negative evidence is not conclusive, the extensive history of rotifer sampling across aquatic and semiterrestrial habitats makes it difficult to suggest new ways to test the cryptic male hypothesis directly (but see [38]). One approach might involve leveraging new data and techniques [58,59] to revisit early attempts at inferring 'sperm-specific' proteins in other rotifers [58–60] and examining their fate in bdelloids.

There are neither males nor sex, but genetic exchange occurs by alternative mechanisms

Another hypothesis is that bdelloid rotifers might exchange or transfer genes within populations in alternative ways that are distinct from outcrossing. For example, perhaps horizontal transfer between homologous loci occurs by uptake of exogenous DNA (for example, during DNA repair after desiccation [40]) or via vectors such as mobile elements (analogous to transformation and transduction, respectively, in bacteria [61]). This hypothesis was initially prompted by the discovery of high levels of distant HGT from non-metazoan sources [40], though whether near HGT within populations would occur by similar means is unknown. Evidence to assess this possibility directly is currently lacking (Box 1), and we know of no successful transformation experiments with bdelloids to date. Frequent HGT might be expected to generate a patchwork of allele-sharing patterns at restricted sets of loci rather than the genome-wide shuffling or sharing expected through outcrossing [38]. Other alternative mechanisms might include parasexual exchange, as in fungi, which can involve the transfer of large genome regions or chromosomes [62], but requires a haploid life stage which is thus far unknown in bdelloids. All these hypotheses are speculative at present, and it is unclear to what extent they might recapitulate theoretical benefits of sex. Nonetheless, even heterodox ideas should be considered, given the puzzle of the missing males.

There are neither males, sex, nor any other kinds of exchange

The final possibility is that both outcrossing and genetic exchange are absent in bdelloid rotifer populations, and signatures interpreted in this light have other explanations. For example, these might include gene duplication and loss, rare recombination between divergent paralogous copies within gene families, uncertainties in haplotype or ancestral recombination reconstruction, and unrepresentative subsampling or filtering from the full set of genomic loci (Box 1). Both studies reporting allele sharing patterns analysed only a fraction of the genome (<10%), so that biases in assembly, variant calling, filtering, and tree inference stages could influence some results. Trees inferred within arbitrary genomic windows are likely to encompass mixed signal from sequence blocks with different histories due to asyngamous recombination (Box 2). This could lead to topological incongruence and apparent allele sharing as an artefact (e.g., Figure S4 in [63]), potentially even without sex. Unexpected patterns of allelic divergence could also arise from broader genome-scale mechanisms, such as further tiers of historic polyploidy, disparities in ploidy between germline and somatic nuclei, or asymmetries of evolutionary rates between haplomes. Genome data from an expanding range of metazoans is revealing diverse genetic systems that could affect signatures in ways that are difficult to anticipate [64].

So far, many studies have adopted a qualitative approach (Box 2), in which allele trees or sharing patterns incompatible with verbal predictions of strict asexuality are taken as evidence of outcrossing or alternative forms of genetic exchange. Further resolution will come partly from chromosome-scale genomic data for more populations and species, but also from applying population genetic models and simulations to generate quantitative predictions about the effects of different genetic systems on the frequency of certain genomic signatures [37,65]. Comparisons

of tree topologies among loci could use ancestral recombination graph (ARG) approaches that infer how genealogical relationships change along the genome due to recombination breakpoints [66–68]. Modelling would enable alternative assumptions, scenarios, and interactions to be defined and quantified, and then establish whether the parameters underpinning these scenarios are compatible with, for example, strict asexuality or rare sex. Philosophically, the Bayesian prior for such tests ought to be uniform: evaluating evidence across a range of scenarios rather than directing the burden of proof towards a particular claim. Whichever way the paradox of males is finally resolved, its investigation is bringing a deeper understanding of the effects of sex, recombination, and genetic exchange in animal genomes.

Why is there so much HGT in bdelloid genomes?

A remarkably high proportion of bdelloid genes apparently have been acquired via HGT from non-metazoan sources. Over 8% of genes share closer sequence identity with non-metazoans than with metazoans, or encode proteins not found in other animals, indicating an unusually high level of non-homologous, inter-kingdom horizontal transfer (denoted 'distant HGT') compared with other animals (Figure 2A). The pattern is consistent across a range of data types (fosmid [40], transcriptomic [32], and genomic [31,34,36]), and among all bdelloid species investigated thus far, while absent from their nearest phylogenetic relatives [32] (but see [59]).

Contamination is ruled out by the unambiguous placement of HGT candidate genes (**HGTcs**) within assembled bdelloid chromosomes [36] (Figure 2C). Alternative evolutionary explanations – such as loss of ancient gene families from all other metazoa except bdelloids, gain of many genes in a single event (e.g., from a parasite or endosymbiont), or convergent evolution of bdelloid genes towards non-metazoan sequence motifs – are difficult to reconcile given the diversity of putative donors from multiple bacterial phyla, plants, fungi, and single-celled eukaryotes (Figure 2B). Most work has focused on non-metazoan sources to date because of the ease of detection against a metazoan background, but the same mechanisms could presumably work for metazoan DNA. The increasing number and diversity of high-quality animal genomes will allow robust phylogenetic estimation of the level of animal DNA that has been acquired.

Unlike cases in prokaryotes, most HGTcs in bdelloids are too highly modified from their closest non-metazoan matches (including acquiring introns and metazoan promoters [40]) to establish a precise origin, and likely descend from ancient acquisitions. Given the challenges and modifications involved in domesticating transferred genes, most retained HGTcs probably benefit the animal. The precise functions of HGTcs are mostly unknown, however, because of the current lack of tools for gene knockdown or modification in bdelloids. Functional annotations and expression patterns nonetheless support roles in processes such as DNA repair [69,70], resistance to fungal pathogens [71], TE silencing [35] and/or gene regulation via epigenetic DNA modification [72], and long-chain fatty acid biosynthesis [73]. Functional annotations include biochemical pathways that are greatly expanded compared with those of other animals, such as carbohydrate metabolism and toxin degradation [32]. Those HGTcs that show degenerate tetraploidy in *A. vaga* (and hence are assumed to be present in the most recent common ancestor of extant bdelloids) are enriched for DNA recombination and ligation functions, which could have contributed to bdelloids' ability to repair **DNA double-stranded breaks (DSBs)** (next section). Future methods for genetic manipulation will be required to test these functions directly.

How HGTcs are acquired is currently unknown. An early hypothesis was that they could be incorporated rarely and accidentally during repair of DSBs after desiccation, perhaps by non-homologous end joining [10,39,40]. This has not been observed directly, however, and bdelloid species that live in fully aquatic habitats and lack measurable survival upon desiccation still have

unique HGTcs acquired after their divergence [36,39], together with a higher density of TEs [35]. Perhaps exogenous DNA is picked up during dynamic rearrangements in bdelloid telomeres and then shuffled to other genomic locations via TE movements or transposon-mediated ectopic recombination. Recently discovered giant retroelements called *Terminons* (exceeding 40 kb in length) may play a role in transfer [74], although their dynamics and effect on HGT remain to be investigated.

Why do bdelloids have so many HGTcs? They might have mechanisms that promote higher rates of arrival of exogenous DNA into their germlines. Alternatively, perhaps the rate of exposure to potential transfers is similar to that in other animals, but bdelloids are more able or under stronger selection to retain them. It is tempting to relate HGT to the other unusual features of bdelloids, namely anhydrobiosis and the lack of males, but comparisons across bdelloid species do not support a strong causal role for desiccation in promoting HGT [34], nor do other anhydrobiotic animals (e.g., tardigrades [75]) show equivalent levels of HGT (though their mechanisms of desiccation tolerance may also differ). Similarly, there is no clear tendency for high levels of HGT in other asexual animals [41], though such cases are often interspersed with close sexual relatives and retain the ability to produce males. Even though the level of HGT from non-metazoan sources is high, the rate of gain appears to be low, estimated as 12.8 gains per million years [39]. This rate is too low to act as a direct replacement for outcrossing, but could be viewed as a slow but steady source of new functions. It is also too low to facilitate direct observation and experimentation into causes of HGT, meaning that future resolution will depend upon phylogenetic and genomic inferences, such as identifying putative cases of recent transfer from a known donor. Sampling of genomes from across the eukaryote tree of life will allow a robust assessment of the 'uniqueness' of high HGT in bdelloids and may reveal cases in other taxa that would shed light on its correlates. For now, bdelloids are an important system to understand the transfer of metabolic and biochemical functions across domains of life.

How does stress tolerance both influence and depend upon genome dynamics?

Bdelloid rotifers have long been recognised as extraordinarily resistant to multiple stressors, including cold, heat, vacuum, and ultraviolet radiation [76]. However, many of these extremophilic capabilities appear to be directly or indirectly related to their tolerance of desiccation, which is an ecologically crucial trait in their temporary water habitats. Among numerous adverse effects, desiccation disrupts cell membranes, produces reactive oxygen species (ROS), and denatures proteins [77]. Particularly relevant to genome dynamics, however, is the potential to cause DSBs. The discovery that bdelloids are extraordinarily resistant to ionising radiation (IR) – a stressor that is not as ecologically salient, but also results in DSBs [19] – led to the development of the DSB-repair hypothesis [10]. This proposes that the ability to repair DSBs caused by desiccation is a key attribute explaining bdelloid stress tolerance that might unite various features of bdelloid genome dynamics (summarised in Figure 3A). For instance, maintenance of homologous chromosomes as templates for DNA repair could explain their collinearity and low divergence [48], while breaks and their repair have been speculated to facilitate HGT via incorporation of exogenous DNA.

Recent imaging and molecular experiments have supported several core elements of this synthesis (Table 1), including short-term repair dynamics, the timing of which appears to differ between somatic and germline cells (Figure 3B,C). A report of chromosome pairing during oogenesis provides a potential mechanism for germline DNA repair [27]. Other parts of the hypothesis, however, especially in relation to evolutionary and genomic consequences of repair (such as for HGT) remain hypothetical or partly contradicted by current evidence (Table 1). One area for future work is to distinguish the effects of IR from those of desiccation. Experiments have focused on IR as much

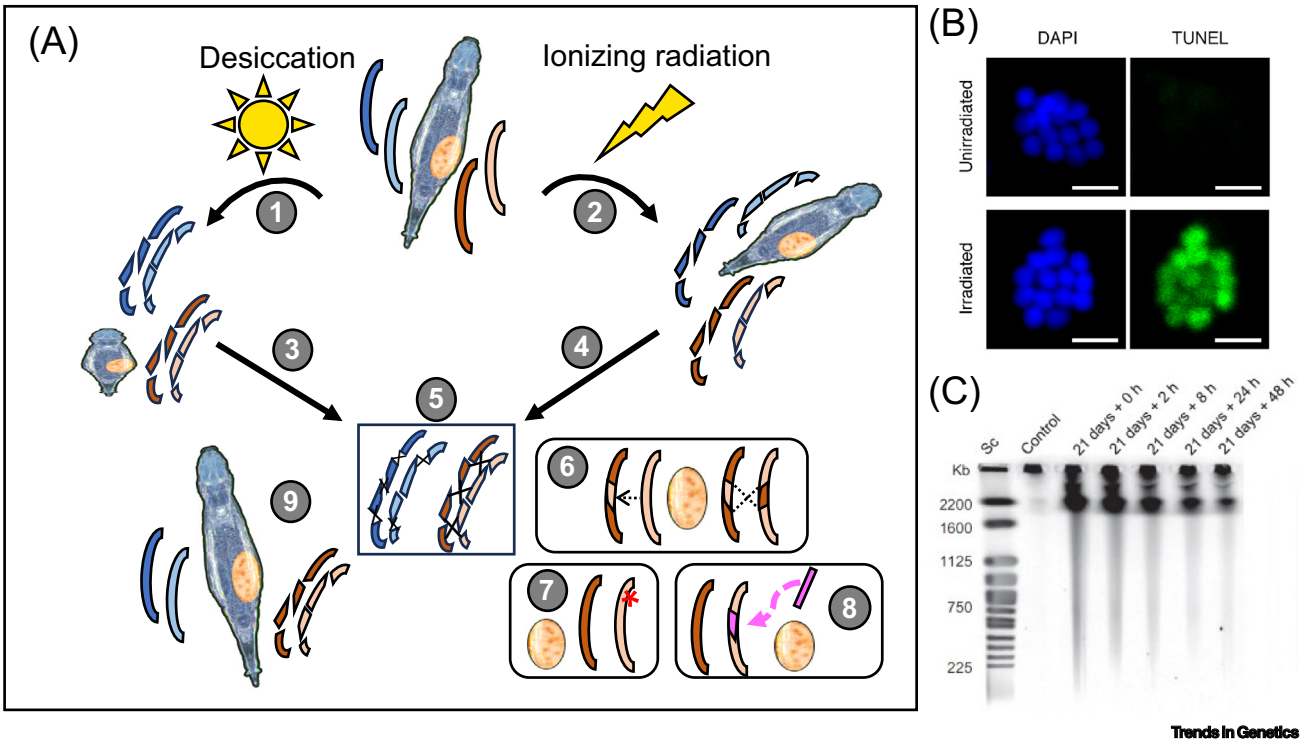

Figure 3. Evidence for key links in the DNA double-strand break (DSB) repair hypothesis and its extensions. (A) The core steps 1–5 propose that repair of DSBs is a key part of bdelloid tolerance of desiccation and ionising radiation; later steps 6–9 associate this process with other phenomena, such as loss of heterozygosity (LoH). The two stressors are shown separately to distinguish evidence in each case, where numbers link to Table 1. Different hypotheses have been proposed for somatic (blue) and germline (orange) cells, with one pair of homologous chromosomes depicted for each. (B) Imaging of primary oocytes in *Adineta vaga* showing DNA (in blue), with DSBs revealed in proton-irradiated animals by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL). Unirradiated animals were desiccated for 72 h, but show no TUNEL signal that would indicate germline DSBs (from [27], scale bar 10 μm). (C) Pulsed-field gel electrophoresis (PFGE) showing repair of fragmented DNA following 21 days of desiccation at different time points post-rehydration (from [26], with permission), together with intact DNA remaining in wells. Abbreviation: DAPI, 4′,6-diamidino-2-phenylindole.

as on desiccation, because it is tractable, recapitulates some of the effects of desiccation, and is interesting for astrobiology, genome stability, and synthetic biology [21,78]. Yet current evidence indicates that the two stressors may not be directly interchangeable (Table 1). A recent study found that only 7% of genes significantly upregulated in response to 14 days of desiccation were also upregulated in response to IR without desiccation [79]. The response to UV radiation seems to differ again in ways that would be worth investigating [26]. Tracking the dynamics of repair and recombination over generational timeframes will also be important, with early work in this area indicating that DSBs may be induced even without external stressors, although desiccation and IR increase their frequency [51].

Evaluation of the importance of DNA repair as a key trait behind desiccation tolerance will require revisiting other aspects of the bdelloid stress response, such as protection against ROS and protein denaturing, compared with other animals. Desiccation tolerance mechanisms might not explain resilience to other stresses; for instance, chemical toxins [32], starvation [80], anoxia, pathogens, and freezing probably require additional pathways [76]. A major question is to what extent non-metazoan genes (whether or not their acquisition results from DSB repair) help to underpin stress tolerance. There is evidence that certain acquired genes play a key role in recovery from desiccation [69], in DNA repair [36,70], and potentially in degradation of chemical toxins [32]. Many horizontally acquired genes are upregulated in response to ionising radiation, though these

Table 1. Evaluation of evidence for the different steps of the DSB-repair hypothesis for tolerance of desiccation and ionising radiation as numbered in Figure 3A

#	Hypothesis or link	Status of evidence
1	Desiccation induces DSBs	Good evidence in other systems [77] and bdelloids. Pulsed-field gel electrophoresis (PFGE) reveals modest DNA degradation, increasing after 21–42 days (*A. vaga*), albeit with limited resolution for larger fragments [26]. Replicated across multiple genera: *Habrotrocha*, *Philodina* [20]. PFGE reflects somatic rather than germline DNA outcomes; no evidence of DSBs in germline after 72 h desiccation (Figure 3C).
2	IR induces DSBs	Well-established radiobiology, strong evidence in many organisms [77], including bdelloids [26]. PFGE reveals massive fragmentation in soma at even modest doses. DSBs also apparent in germline cells (primary oocytes) [27] (Figure 3C).
3	Desiccation-induced DSBs are repaired in somatic and germline cells	Direct evidence for DSB repair in one PFGE (Figure 3B), 0–48 h after rehydration. Modest differences between pre- and post-repair DNA, mostly somatic DNA [26]. Intact high-molecular-weight DNA is also present throughout. Nuclear staining indicates no DNA synthesis in somatic cells or developing eggs after 24 h desiccation, but PFGE suggests that damage is not extensive. Germline DNA damage from longer desiccations in nature must be successfully repaired. DNA repair pathways strongly upregulated after desiccation, at least in somatic cells [69]
4	IR-induced DSBs are repaired in somatic and germ cells	Strong PFGE evidence for somatic cell DNA repair in response to various IR sources [20,21,26]. Recovery of DNA sequence (not just size) shown by restriction profiling [27]. Staining reveals rapid DNA synthesis during somatic cell repair, but not in primary oocytes; germline repair is delayed until oocyte maturation and perhaps beyond [51]. DNA repair genes strongly upregulated after irradiation and expressed in somatic nuclei [70], but only a minority of IR-upregulated genes (7–18%) overlap with the desiccation response [79]
5	DSB repair involves pairing of homologous chromosomes	Chromosomes are homologous [36], and bivalents form during normal oogenesis [27], but pairing is not observed immediately after DSB damage occurs, nor in somatic cells. Repair genes upregulated in response to desiccation belong primarily to the non-homologous end-joining and break excision pathways [69,79], rather than homologous recombination as in other systems [77]. Germline damage must be repaired because an original restriction profile is restored in F1 [27], and this may involve different pathways from somatic cells [51]
6	DSB repair causes LoH and/or recombination	Modified meiosis with pairing might be linked to LoH irrespective of external stressors, as seen in hydrated lines [36,51]. Desiccation- or IR-induced DSBs may accelerate LoH, but are not required. Other observations also are inconsistent with a requirement for desiccation (e.g., non-desiccating rotifers have higher LoH [34]; other non-desiccating asexuals show meiotic pairing and LoH)
7	DSB repair aids selection against deleterious mutations	Unknown, despite the intuition that, if DNA repair were associated with recombination, it could alleviate some of the predicted costs of asexuality [31,85]. Unclear whether desiccation facilitates selection in the longer term or is mutagenic (mutation accumulation experiments are needed). Older studies suggest slightly higher or equal deleterious substitution rates in bdelloids versus monogononts [86]
8	Desiccation and DSB repair promote HGT	No evidence at present for either near or distant HGT. Other desiccating animals lack HGT, or elevated incorporation of non-nuclear (e.g., mitochondrial) DNA [87]. Non-desiccating bdelloids still have HGT gains [39]. No direct observations of HGT via desiccation in experiments (may be too rare)
9	DSBs and their repair underpin bdelloid stress tolerance	Repair of DSB is necessary but probably not sufficient. Desiccation duration correlates with survival and so does DNA damage [26], but survival is more robust after proton irradiation despite far more DNA breaks than 42 days of desiccation. UV radiation, despite not causing DNA damage as severe as proton radiation, strongly decreases survival, presumably in other ways (e.g., ROS, mutations, protein damage). Different types of IR cause the same DSB patterns at the same doses [21], but show differently shaped survival and fecundity curves. Desiccation-intolerant *Rotaria* spp. survive radiation as well as desiccating bdelloids (although their fertility is more impacted) and have similar capacity for repairing the DSBs as desiccating bdelloids [20]. Mitigating other harms of desiccation may be at least as important in survival as DSBs [28], such as protection of proteins from ROS [77]

are not overrepresented relative to the genomic baseline, as is seen following exposure to desiccation [79] and pathogens [71]. There is scope to measure the contribution of HGT to stress-induced expression more generally. Further transcriptomic experiments (ideally combined with genetic manipulation) and comparisons with non-desiccating rotifers, as well as other stress-tolerant animals such as tardigrades and nematodes, will help to clarify the importance of HGT for bdelloid resilience. Do acquired genes replace or supplement other pathways, and are they more strongly associated with some stressors than others? Valuable insights will also arise from investigating how shared animal genes, which still greatly outnumber HGTcs, have adapted to provide ecological resilience in a group far removed from well-studied metazoan models.

Key figure

Diagram of potential interactions between four elements of bdelloid biology, and their potential influence on genome dynamics

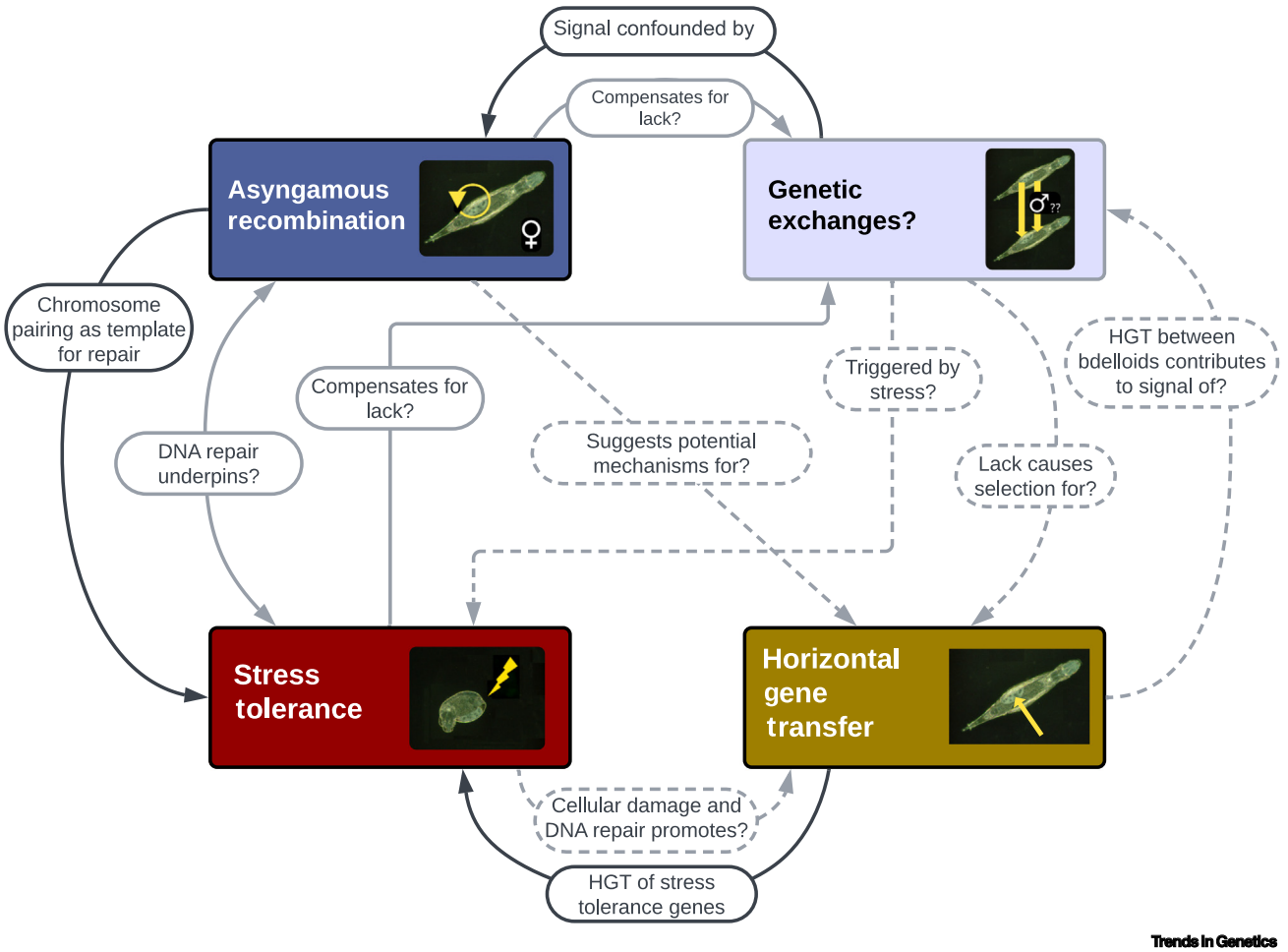

Figure 4. Some hypothetical links from this review and the broader literature are shown, but this is not exhaustive. Arrows and boxes in black indicate links with clearer evidence, grey indicates emerging but partially incomplete evidence, and dashed grey indicates connections or phenomena we view as more ambiguous or hypothetical at the time of writing. Abbreviation: HGT, horizontal gene transfer.

Concluding remarks

The combination of more and better genomes with improved cytological imaging has led to a recent step change in knowledge of the genetic processes at work in bdelloid rotifers. Our view of the diversity of reproductive modes has also moved beyond a binary classification into sexual or asexual [64] towards an understanding of how mechanisms of genetic transmission and recombination interact to produce long- and short-term genomic consequences. Whatever resolution is eventually reached, efforts to interpret the genetic system of bdelloids have already contributed to a greater understanding of animal genome evolution. Some notes of caution and caveats exist for the remaining puzzles. Most experimental and genomic work has focused on one lab-cultivated species, *A. vaga*, and we should not assume that all bdelloids are the same: they have varied lifestyles and habitats, and have diverged over tens of millions of years. Other species show hints of departure from strictly diploid organisation of karyotypes [44]. Finally, because bdelloids are unusual, it is tempting to expect or accept unusual conclusions about their biology. Care is needed to provide evidence for each process and the hypothesised links between various 'strange' features (Figure 4, Key figure). Key future advances (see Outstanding questions) will come from methods of genetic manipulation to establish the basis of bdelloid phenotypes; from multispecies population genomics based on single-individual long-read sequencing; from structural, transcriptional, and phylogenetic comparisons; and from interpreting genomic patterns using a quantitative, simulation-based framework.

Outstanding questions

How frequently does recombination bring together DNA from different parental genomes, relative to recombination within a parthenogenetic line of descent?

Without direct observations, what constitute robust signatures of outcrossing against a background of asyngamous recombination and reconstruction artefacts?

Over what genomic scale does asyngamous recombination occur during parthenogenesis? If it typically leads to loss of heterozygosity, how is heterozygosity restored or maintained?

If genetic exchange does occur, is this by cryptic males, or some other mechanism? If outcrossing is implied by genomic data, what do the males or hermaphrodites look like and how were they missed?

Is metazoan DNA captured by HGT and, if so, more or less than from other sources? Do similar or different mechanisms transfer homologous DNA within or between bdelloid populations compared with heterologous sequences?

How is exogenous DNA first acquired into the germline? Does it involve DNA repair, telomeric rearrangements, or TEs? What source pool of environmental DNA are bdelloids exposed to? How do they domesticate and retain DNA from non-metazoan sources into functioning, expressed genes?

What are HGTcs used for? Have they led to major shifts in the biochemical and functional capabilities of bdelloids, such as stress tolerance?

Does desiccation shape genome structure and content through its effects on DSBs and associated repair mechanisms? How important are DNA repair mechanisms and HGTcs for desiccation and other stresses in bdelloids, relative to other protection mechanisms? How is mitochondrial DNA repaired or protected from DSBs?

Does desiccation tolerance promote HGT and asyngamous recombination, or are these processes largely independent?

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Declaration of interests

The authors have no conflicts of interest to declare.

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Taken together, how do the aforementioned mechanisms interact to generate and preserve genomic variation within and between bdelloid populations, and promote adaptation and diversification? Specifically, how would these processes influence the presumed costs and benefits of life without males, such as removing deleterious mutations, maintaining beneficial gene combinations, and responding to changing selection?

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