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Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*

Jean-François Flot[1,2,3,4,5,6], Boris Hespeels[1,2], Xiang Li[1,2], Benjamin Noel[3], Irina Arkhipova[7], Etienne G. J. Danchin[8,9,10], Andreas Hejnol[11], Bernard Henrissat[12], Romain Koszul[13], Jean-Marc Aury[3], Valérie Barbe[3], Roxane-Marie Barthélémy[14], Jens Bast[15], Georgii A. Bazykin[16,17], Olivier Chabrol[14], Arnaud Couloux[3], Martine Da Rocha[8,9,10], Corinne Da Silva[3], Eugene Gladyshev[7], Philippe Gouret[14], Oskar Hallatschek[6,18], Bette Hecox-Lea[7,19], Karine Labadie[3], Benjamin Lejeune[1,2], Oliver Piskurek[20], Julie Poulain[3], Fernando Rodriguez[7], Joseph F. Ryan[11], Olga A. Vakhrusheva[16,17], Eric Wajnberg[8,9,10], Bénédicte Wirth[14], Irina Yushenova[7], Manolis Kellis[21], Alexey S. Kondrashov[16,22], David B. Mark Welch[7], Pierre Pontarotti[14], Jean Weissenbach[3,4,5], Patrick Wincker[3,4,5], Olivier Jaillon[3,4,5,21]* & Karine Van Doninck[1,2]*

Loss of sexual reproduction is considered an evolutionary dead end for metazoans, but bdelloid rotifers challenge this view as they appear to have persisted asexually for millions of years[1]. Neither male sex organs nor meiosis have ever been observed in these microscopic animals: oocytes are formed through mitotic divisions, with no reduction of chromosome number and no indication of chromosome pairing[2]. However, current evidence does not exclude that they may engage in sex on rare, cryptic occasions. Here we report the genome of a bdelloid rotifer, *Adineta vaga* (Davis, 1873)[3], and show that its structure is incompatible with conventional meiosis. At gene scale, the genome of *A. vaga* is tetraploid and comprises both anciently duplicated segments and less divergent allelic regions. However, in contrast to sexual species, the allelic regions are rearranged and sometimes even found on the same chromosome. Such structure does not allow meiotic pairing; instead, we find abundant evidence of gene conversion, which may limit the accumulation of deleterious mutations in the absence of meiosis. Gene families involved in resistance to oxidation, carbohydrate metabolism and defence against transposons are significantly expanded, which may explain why transposable elements cover only 3% of the assembled sequence. Furthermore, 8% of the genes are likely to be of non-metazoan origin and were probably acquired horizontally. This apparent convergence between bdelloids and prokaryotes sheds new light on the evolutionary significance of sex.

With more than 460 described species[4], bdelloid rotifers (Fig. 1) represent the highest metazoan taxonomic rank in which males, hermaphrodites and meiosis are unknown. Such persistence and diversification of an ameiotic clade of animals are in contradiction with the supposed long-term disadvantages of asexuality, making bdelloids an 'evolutionary scandal'[5]. Another unusual feature of bdelloid rotifers is their extreme resistance to desiccation at any stage of their life cycle[6], enabling these microscopic animals to dwell in ephemeral freshwater habitats such as mosses, lichens and forest litter; this ability is presumably the source of their extreme resistance to ionizing radiation[7].

We assembled the genome of a clonal *A. vaga* lineage into separate haplotypes with a N_{50} of 260 kilobases (kb) (that is, half of the assembly was composed of fragments longer than 260 kb). Assembly size was 218 megabases (Mb) but 26 Mb of the sequence had twice the average sequencing coverage, suggesting that some nearly identical regions were not resolved during assembly (Supplementary Fig. 3); hence, the total genome size is likely to be 244 Mb, which corresponds to the estimate obtained independently using fluorometry (Supplementary Note C2). Annotation of the complete assembly (including all haplotypes) yielded 49,300 genes. Intragenomic sequence comparisons revealed numerous homologous blocks with conserved gene order (colinear regions). For each such block we computed the per-site synonymous divergence (Ks) and a colinearity metric defined as the fraction of colinear genes. Colinear blocks fell into two groups (Fig. 2a): a group characterized by high colinearity and low average synonymous divergence, and a group characterized by lower colinearity and higher synonymous divergence. The presence of two classes of colinear blocks is consistent with a tetraploid structure comprised of alleles (recent homologues) and ohnologues (ancient homologues formed by genome duplication). Allelic pairs of coding sequences are on average 96.2%

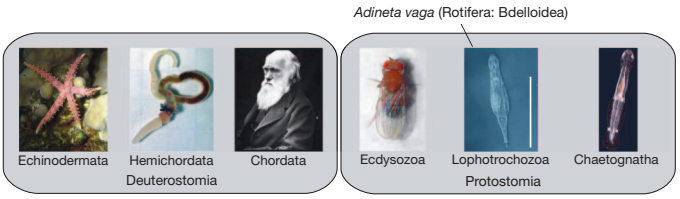

Figure 1 | Position of bdelloid rotifers among metazoans. Bdelloid rotifers ('leech-like wheel-bearers') are a clade of microscopic animals (scale bar, 100 μm) within the phylum Rotifera. Photographs of Hemichordata (*Saccoglossus*), Chordata (*Homo*) and Ecdysozoa (*Drosophila*) courtesy of David Remsen (MBL), John van Wyhe (http://darwin-online.org.uk) and André Karwath, respectively.

[1]University of Namur, Department of Biology, URBE, Laboratory of Evolutionary Genetics and Ecology, 5000 Namur, Belgium. [2]Namur Research Institute for Life Sciences (NARILIS), 5000 Namur, Belgium. [3]CEA-Institut de Génomique, GENOSCOPE, Centre National de Séquençage, 2 rue Gaston Crémieux, CP5706, 91057 Evry Cedex, France. [4]Université d'Evry, UMR 8030, CP5706, 91057 Evry Cedex, France. [5]Centre National de la Recherche Scientifique (CNRS), UMR 8030, CP5706, 91057 Evry Cedex, France. [6]Max Planck Institute for Dynamics and Self-Organization, Biological Physics and Evolutionary Dynamics, Bunsenstraße 10, 37073 Göttingen, Germany. [7]Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA. [8]INRA, UMR 1355 ISA, Institut Sophia Agrobiotech, 400 route des Chappes, 06903 Sophia-Antipolis, France. [9]CNRS, UMR 7254 ISA, Institut Sophia Agrobiotech, 400 route des Chappes, 06903 Sophia-Antipolis, France. [10]Université de Nice Sophia-Antipolis, UMR ISA, Institut Sophia Agrobiotech, 400 route des Chappes, 06903, Sophia-Antipolis, France. [11]Sars International Centre for Marine Molecular Biology, University of Bergen, 5008 Bergen, Norway. [12]Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille University, CNRS UMR 7257, 13288 Marseille, France. [13]Groupe Spatial regulation of genomes, CNRS UMR 3525, Department of Genomes and Genetics, Institut Pasteur, 75724 Paris, France. [14]LATP UMR-CNRS 7353, Evolution Biologique et Modélisation, Aix-Marseille University, 13331 Marseille cedex 3, France. [15]J.F. Blumenbach Institute of Zoology and Anthropology, University of Göttingen, 37073 Göttingen, Germany. [16]Department of Bioengineering and Bioinformatics, M.V. Lomonosov Moscow State University, Leninskye Gory 1-73, Moscow, 119991, Russia. [17]Institute for Information Transmission Problems of the Russian Academy of Sciences (Kharkevich Institute), Bolshoi Karetny pereulok 19, Moscow, 127994, Russia. [18]Department of Physics, University of California, Berkeley, California 94720, USA. [19]Department of Biology, Northeastern University, Boston, Massachusetts 02115, USA. [20]Courant Research Centre Geobiology, Georg-August-Universität Göttingen, Goldschmidtstraße 3, Göttingen 37077, Germany. [21]MIT Computer Science and Artificial Intelligence Laboratory, Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02139, USA. [22]Life Sciences Institute and Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, Michigan 48109-2216, USA.
*These authors contributed equally to this work.

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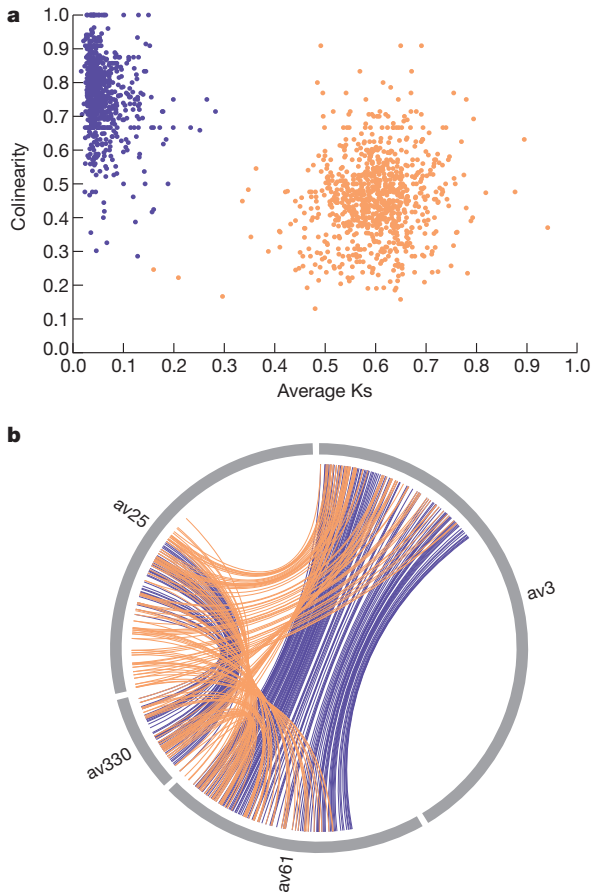

Figure 2 | A locally tetraploid genome. **a**, Analysis of intragenomic synteny reveals two groups of colinear regions: alleles (in violet, regions characterized by a high fraction of colinear genes and low average Ks, that is, synonymous divergence) and ohnologues (in orange, with lower colinearity but higher Ks). **b**, Example of a genomic quartet of four scaffolds: allelic gene pairs are connected with violet curves and ohnologous gene pairs with orange curves.

identical at the nucleotide level (median = 98.6%) versus 73.6% (median = 75.1%) for ohnologous pairs. Nearly 40% (84.5 Mb) of the assembled genome sequence is organized in quartets of four homologous regions A_1, A_2, B_1 and B_2, of which A_1–A_2 and B_1–B_2 are two pairs of alleles and As are ohnologous to Bs[8] (Fig. 2b).

We found evidence of genomic palindromes up to 705 kb in length and involving up to 148 genes. The *A. vaga* genome contains at least 17 such palindromic regions (Fig. 3a) reminiscent of those reported in the Y chromosomes of primates[9]. In all 17 cases, the arms of the palindromes present the colinearity and divergence signatures of allelic regions and do not have other allelic duplicates in the assembly, suggesting that they arose by inter-allelic rearrangements rather than by local duplications. In addition to these 17 inverted repeats, we observed three direct repeats that present the signatures of allelic blocks and involve up to 50 genes (Fig. 3a). The cumulative length of the assembly fragments (scaffolds) bearing these 20 allelic rearrangements is 7.5 Mb or 3.5% of the genome sequence. Allelic regions that are found on the same chromosome clearly cannot segregate during meiosis. Moreover, we found hundreds of colinearity breakpoints between allelic regions, and the total length of the scaffolds that have no full-length homologue in the assembly due to these breakpoints exceeds 109 Mb or 51% of the genome assembly (including 91 of the 100 largest scaffolds, Fig. 3b and Supplementary Fig. 10). As a result, it is impossible to split the assembled genome of *A. vaga* into haploid sets: the apparent ploidy level of *A. vaga* is scale-dependent, with a tetraploid structure at gene scale versus chromosome-scale haploidy. Such relaxation of constraints on genome structure is reminiscent of other mitotic lineages such as cancer cells[10] and somatic tissues[11].

It has been proposed that, in the absence of meiosis, alleles accumulate mutations independently from one another, to the point that ancient asexuals may harbour genome-wide allele sequence divergence (ASD)[12] larger than inter-individual differences (the so-called 'Meselson effect'). However, the average inter-allelic divergence of *A. vaga* is only 4.4% at the nucleotide level (3% when looking at synonymous divergence), which falls in the upper range reported for sexually reproducing species[13]. The absence of genome-wide ASD could be explained by low mutation rates and/or by frequent mitotic recombination (such as gene conversion resulting from DNA repair)[12]. Although there is no evidence of reduced mutation rates in bdelloid rotifers compared with their cyclically sexual sister clade the monogononts[14], we found strong signatures

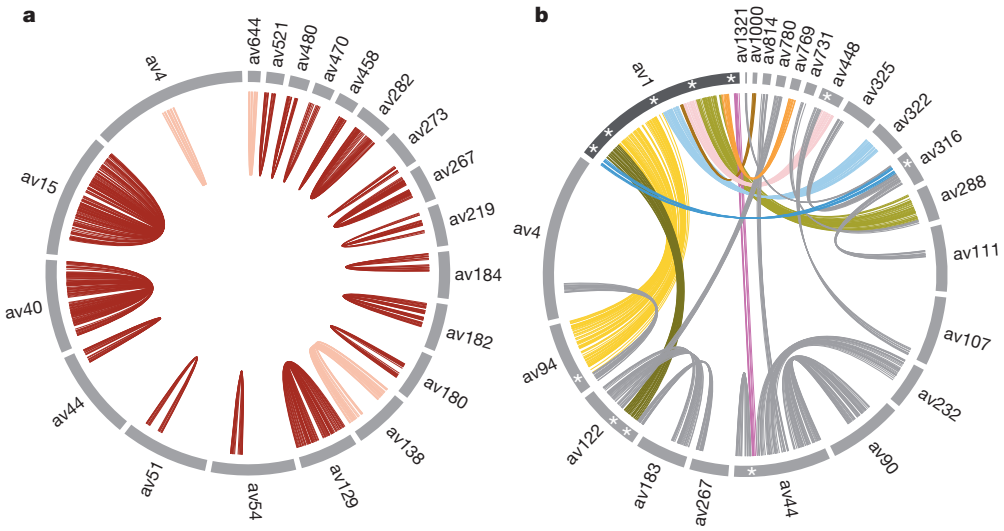

Figure 3 | A genome structure incompatible with conventional meiosis. **a**, In twenty cases, allelic regions are found to occur on the same chromosome. All curves shown connect allelic gene pairs. On three scaffolds both allelic regions have the same orientation (direct repeats, in pink), whereas on the seventeen other scaffolds they are inverted (palindromes, in red). **b**, Local colinearity between alleles does not extend to chromosome scale. Colours are arbitrary and only allelic gene pairs are represented. Asterisks highlight colinearity breakpoints between scaffold av1 and its allelic partners av44, av94, av122, av316 and av448. Further examples for other scaffolds are shown on Supplementary Fig. 10.

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of recent gene conversion events in the distribution of identity track lengths, that is, distances between consecutive mismatches (Fig. 4a and Supplementary Note E1). We calculated that the probability that a given base in the genome experiences gene conversion is at least one order of magnitude greater than its probability to mutate (Supplementary Note E1), suggesting that homologous regions in the genome of *A. vaga* undergo concerted evolution[15]. Homogenization through gene conversion may either expose new mutations to selection by making them homozygous or remove them as they get overwritten with the other allelic version (Fig. 4b), thereby slowing Muller's ratchet (that is, the irreversible accumulation of detrimental mutations in asexual populations of finite sizes, Supplementary Note E2 and Supplementary Fig. 11).

Over 8% of the genes of *A. vaga* are much more similar to non-metazoan sequences in GenBank than to metazoan ones (AI log score > 45 (ref. 16), Supplementary Note E4) and were therefore probably acquired through horizontal gene transfer (HGT). This class of genes has significantly fewer introns per kilobase of coding sequence compared with probable core metazoan genes (AI ≤ −45, Supplementary Table 2). More than 20% of genes with AI > 45 are found in quartets (groups of four homologous copies in conserved syntenic regions) and were therefore probably incorporated into the rotifer genome before the establishment of tetraploidy, which itself pre-dates the divergence

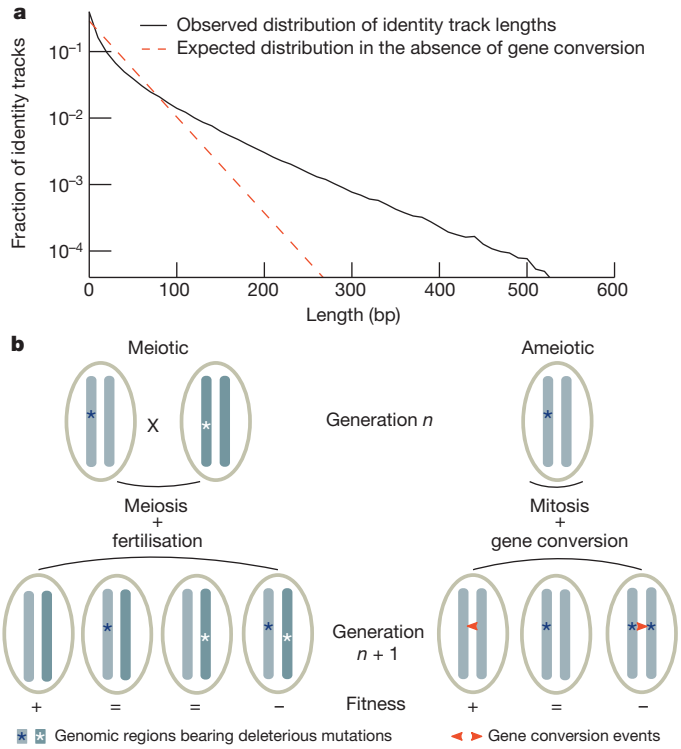

Figure 4 | Gene conversion and its evolutionary consequences in ameiotic organisms. **a**, Evidence for gene conversion between allelic regions. If we suppose that mutations happen at random in a Poisson process of parameter $1/M$ (where M is the average distance between mutations), then the distance between two consecutive mismatches follows a negative exponential distribution where the proportion of identity tracks of length x equals $e^{-x/M}/M$. Comparison of the observed distribution of identity track lengths with this theoretical distribution reveals a deficit of short tracks and an excess of long tracks, as expected in case of gene conversion. The same pattern was observed when gene-coding regions were excluded from the analysis (data not shown), thereby ruling out a confounding effect of selection. **b**, In sexual organisms, meiotic recombination can generate offspring with fewer or more deleterious mutations (hence increasing or decreasing fitness) than the previous generation. The same outcome is expected in ameiotic organisms that experience gene conversion: a deleterious allele may be overwritten by a beneficial or neutral one, resulting in an increase in fitness, or may overwrite it, resulting in decreased fitness.

of extant bdelloid families[8]. The higher the number of copies of a putative HGT gene, the higher its number of introns and the closer its guanine–cytosine (GC) content to the *A. vaga* genome average (Supplementary Fig. 22), which suggests that these parameters reflect the age of acquisition. We also noticed signatures of possibly very recent HGTs: 60 genes with AI > 45 are present in only one copy (with normal coverage), have no intron and have a GC content that is more than 1% above or below the genome average (the same scaffolds also bear genes of probable metazoan origin with AI < 0). In summary, there seems to be an ancient but still ongoing process of HGT at a level comparable to some bacteria[17].

Some theories predict that transposable elements should be either absent from the genomes of asexuals[18] or undergo unrestrained expansion after the switch to asexuality, potentially leading to species extinction unless transposable element proliferation is prevented[19]. We found that transposable elements cover about 3% of the *A. vaga* genome, which is less than the percentage reported in most other metazoans (including the genome of the obligate parthenogenetic nematode *Meloidogyne incognita*, 36% of which is made up of repetitive elements[20]). Another surprising feature is the high diversity of transposable-element families and the extremely low copy numbers observed for each of them (Supplementary Table 3). Out of 255 families, the overwhelming majority (209) are represented by only one or two full-length copies (for 24 families, no full-length copies could be identified), and for each full-length copy there are, on average, only about ten times as many transposable-element fragments. This relatively low abundance of decayed copies and the fact that long-terminal-repeat (LTR) retrotransposons have identical or nearly identical LTRs (Supplementary Table 4) suggest that most low-copy-number families represent recent arrivals. This is consistent with an ongoing process of acquisition of transposable elements by HGT.

This hypothesis is further supported by the significantly higher density of transposable elements observed around HGTs and vice-versa (Supplementary Note E5). If *A. vaga* has been acquiring transposable elements by HGT, a question that arises is what keeps their number lower than in most other metazoans. Many fragmented copies have apparently been formed through microhomology-mediated deletions. Excision of LTR retrotransposons has also been occurring through LTR–LTR recombination, leaving behind numerous solo LTRs: for example, two *Juno1* insertions, *Juno1.1* and *Juno1.2*, which were present as full-length copies in the 2006 *A. vaga* fosmid library[21], exist in the current assembly only as solo LTRs (in the same genomic environments and with the same target site duplications). Finally, there is evidence for expansion and diversification of the RNA-mediated silencing machinery. In addition to Dicer1 proteins, which are shared by all metazoans, *A. vaga* possesses a deep-branching Dicer-like clade with uncertain taxonomic placement (Supplementary Fig. 20). The Argonaute/Piwi and RNA-directed RNA polymerase (RdRP) families are also expanded (Supplementary Figs 18 and 19). It is plausible that these proteins participate in epigenetic silencing of transposable elements (as was recently observed for single-copy transgenes in *Caenorhabditis elegans*[22]), thereby preventing horizontally transferred transposable elements from multiplying upon arrival.

Overall, the genome of *A. vaga* comprises more genes than usually reported for metazoans (Supplementary Note F2), as its haplotypes were assembled separately. Even taking this into account, the gene repertoire of *A. vaga* features expansion of several gene families. For example, the genome of *A. vaga* comprises 284 homeobox superclass genes, mostly found in four copies (quartets) but not organized in clusters; very few ohnologues have been lost, resulting in more homeobox genes than in any other metazoan genome sequenced (Supplementary Note F5). Genes putatively related to oxido-reduction processes are substantially more abundant in *A. vaga* than in other metazoan species, and most of the corresponding genes appear to be constitutively expressed (Supplementary Table 9). This is consistent with the recent report of an effective antioxidant protection system

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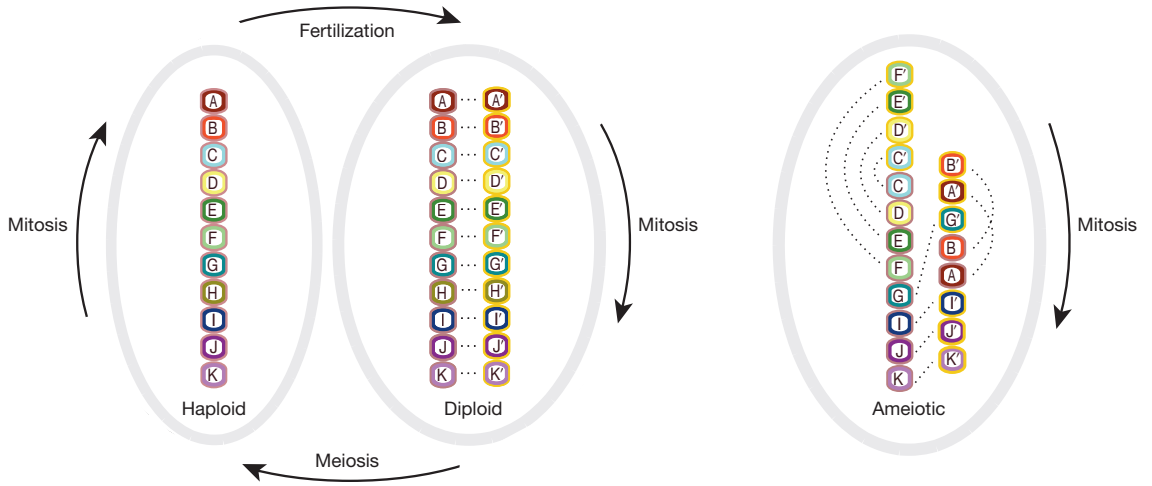

Figure 5 | Meiotic versus ameiotic genome structures. Genes are represented with letters, and dashed lines connect allelic gene pairs. A meiotic genome (left) alternates between a haploid phase (in which a single allele of each gene is present) and a diploid phase (in which the genes are present in two allelic versions arranged colinearly on homologous chromosomes). In the ameiotic genome of *A. vaga* (right), alleles are distributed in blocks that are shuffled across chromosomes, resulting notably in intrachromosomal repeats (direct or inverted). As a consequence, chromosomes have no homologues and cannot be paired.

in bdelloid rotifers[23]. Carbohydrate-active enzymes (CAZymes) in the genome of *A. vaga* are also notably diverse and abundant, with 1,075 genes falling into 202 characterized families. With 623 glycoside hydrolases (involved in the hydrolysis of sugar bonds) and 412 glycosyltransferases (responsible for building sugar bonds), the CAZyme richness of *A. vaga* ranks highest among metazoans and is only comparable to some plants such as poplars[24]. *A. vaga* has the richest repertoire of glycoside hydrolases of any organism sequenced so far, hinting at a diversity of feeding habits; 52% of the CAZymes have an AI > 45 and were therefore probably acquired through horizontal gene transfer.

A. vaga has lost 1,250 genes compared with the inferred last common ancestor of Protostomia, the genome of which comprised at least 7,844 unique protein-coding genes (Supplementary Note E6). A total of 137 PFAM domains typically present in metazoans could not be detected in the assembled genome sequence (Supplementary Data 10). Of particular interest are missing domains involved in reproductive processes (Supplementary Note F1); for example, the *Zona pellucida*-like domain (notably found in sperm-binding proteins[25]) is present in an average of 36 copies in metazoan genomes but is absent in *A. vaga*. In contrast, we found multiple copies of most metazoan genes involved in DNA repair and homologous recombination, including a considerably divergent *Spo11* but no *Rad52* and *Msh3*.

To conclude, our analysis of a lineage of the bdelloid rotifer *Adineta vaga* reveals positive evidence for asexual evolution: its genome structure does not allow pairing of homologous chromosomes and therefore seems incompatible with conventional meiosis (Fig. 5). However, we cannot rule out that other forms of recombination occur in bdelloid populations in ways that do not require homologous pairing, such as parasexuality[26]. The high number of horizontally acquired genes, including some seemingly recent ones, suggests that HGTs may also be occurring from rotifer to rotifer. It is plausible that the repeated cycles of desiccation and rehydration experienced by *A. vaga* in its natural habitats have had a major role in shaping its genome: desiccation presumably causes DNA double-strand breaks, and these breaks that allow integration of horizontally transferred genetic material also promote gene conversion when they are repaired. Hence, the homogenizing and diversifying roles of sex may have been replaced in bdelloids by gene conversion and horizontal gene transfer, in an unexpected convergence of evolutionary strategy with prokaryotes.

METHODS SUMMARY

Genomic DNA was extracted from laboratory cultures of a clonal *A. vaga* lineage and shotgun-sequenced using 454 and Illumina platforms at respective coverage of 25 and 440 times (using both single reads and mate reads from inserts up to 20 kb). The 454 reads were assembled into contigs using MIRA[27]; the contigs obtained were corrected using single Illumina reads and linked into scaffolds using paired Illumina reads[28] (Supplementary Table 1). We annotated protein-coding genes by integrating evidence from RNA sequencing, *ab initio* predictions and comparison with UniProt. Most synteny and Ka/Ks (non-synonymous divergence/synonymous divergence) analyses were performed using the package MCScanX[29] and synteny plots were drawn using Circos[30].

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Supplementary Information is available in the online version of the paper.

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Author Information The sequencing reads and assembly are available at the Sequence Read Archive (accessions ERP002115 and SRP020364 for DNA, ERP002474 and SRP020358 for cDNA) and at the European Nucleotide Archive (accession CAWI000000000), respectively. The assembly and annotation can be browsed and downloaded at http://www.genoscope.cns.fr/adineta, whereas the result of the orthology analysis is accessible at http://ioda.univ-provence.fr/. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to O.J. (ojaillon@genoscope.cns.fr or ojaillon@mit.edu), J.F.F. (jean-francois.flot@ds.mpg.de) or K.V.D. (karine.vandoninck@fundp.ac.be).

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