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Kraaijeveld, K.; Zwanenburg, B.; Hubert, B.; Vieira, C.; Pater, B.S. de; Alphen, J.J.M. van; ... ; Knijff, P. de

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FROM THE COVER

Transposon proliferation in an asexual parasitoid

KEN KRAAIJEVELD,*† BRECHTJE ZWANENBURG,† BENJAMIN HUBERT,‡ CRISTINA VIEIRA,‡ SYLVIA DE PATER,† JACQUES J. M. VAN ALPHEN,§ JOHAN T. DEN DUNNEN* and PETER DE KNIJFF*

*Department of Human Genetics, Leiden University Medical Center S4-P, PO Box 9600, 2300 RC Leiden, The Netherlands, †Institute of Biology, Leiden University, PO Box 9505, 2300 RA Leiden, The Netherlands, ‡Université de Lyon, F-69000, Lyon; Université Lyon 1; CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622, Villeurbanne, France, §IBED, University of Amsterdam, PO Box 94248, 1090 GE Amsterdam, The Netherlands

Abstract

The widespread occurrence of sex is one of the most elusive problems in evolutionary biology. Theory predicts that asexual lineages can be driven to extinction by uncontrolled proliferation of vertically transmitted transposable elements (TEs), which accumulate because of the inefficiency of purifying selection in the absence of sex and recombination. To test this prediction, we compared genome-wide TE load between a sexual lineage of the parasitoid wasp *Leptopilina clavipes* and a lineage of the same species that is rendered asexual by *Wolbachia*-induced parthenogenesis. We obtained draft genome sequences at 15–20× coverage of both the sexual and the asexual lineages using next-generation sequencing. We identified transposons of most major classes in both lineages. Quantification of TE abundance using coverage depth showed that copy numbers in the asexual lineage exceeded those in the sexual lineage for DNA transposons, but not LTR and LINE-like elements. However, one or a small number of *gypsy*-like LTR elements exhibited a fourfold higher coverage in the asexual lineage. Quantitative PCR showed that high loads of this *gypsy*-like TE were characteristic for 11 genetically distinct asexual wasp lineages when compared to sexual lineages. We found no evidence for an overall increase in copy number for all TE types in asexuals as predicted by theory. Instead, we suggest that the expansions of specific TEs are best explained as side effects of (epi)genetic manipulations of the host genome by *Wolbachia*. Asexuality is achieved in a myriad of ways in nature, many of which could similarly result in TE proliferation.

Keywords: asexual reproduction, next-generation sequencing, transposable elements, *Wolbachia*

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Introduction

Transposable elements (TEs) are a ubiquitous component of the genomes of all living organisms and have important effects on genome stability, mutation rates, gene expression and other processes (Lankenau & Volff 2009). TEs are able to replicate independently of and faster than the host genome and accumulate in large numbers unless controlled by natural selection or host suppression mechanisms. Although TE activity may sometimes provide the host with beneficial genetic variation, the majority of TE insertions are thought to be deleterious to host fitness. Hosts will thus be under selection to curb TE proliferation.

The reproductive mode of the host is expected to have profound effects on the dynamics of TEs in their genomes. Sexual reproduction provides mechanisms both for the spread of TEs to new genotypes and for the containment of TE accumulation (Bestor 1999; Wright & Schoen 1999; Wright & Finnegan 2001; Nuzhdin & Petrov 2003; Arkhipova & Meselson 2005; Dolgin & Charlesworth 2006). Sexual recombination uncouples the fate of a TE from that of the genomic background and thus selects for selfish TE behaviour. At the same time, sexual recombination increases the efficacy of natural selection through the purging of highly loaded genotypes, thereby keeping the number

Correspondence: Ken Kraaijeveld, Fax: +31 71 526 8285;
E-mail: ken@kenkraaijeveld.nl

of TE copies in the population in check (Dolgin & Charlesworth 2006). Furthermore, several cellular mechanisms that suppress TE activity are geared towards meiosis or mating, including DNA methylation and RNA interference (RNAi) (Wang *et al.* 2010). When a population switches from sexual to asexual reproduction, it inherits selfish TEs, but loses the ability to generate less loaded genotypes. Depending on the mechanism through which asexuality is achieved, it may also lose some or all of the cellular mechanisms that prevent TE accumulation. Asexual taxa are thus predicted to accumulate TE copies, which may ultimately drive their extinction (Arkhipova & Meselson 2005; Dolgin & Charlesworth 2006).

Studies addressing this hypothesis have been hampered by the lack of suitable model systems and the limited numbers of TEs that could be screened. Consequently, the evidence has remained inconclusive. For example, certain TEs segregate at higher frequencies in the self-fertilizing *Caenorhabditis elegans* and *Arabidopsis thaliana* than in a cross-fertilizing-related species (Wright *et al.* 2001, Dolgin *et al.* 2008; Lockton & Gaut 2010). On the other hand, obligate parthenogenetic populations of *Daphnia pulex* appear to have lower copy numbers of both DNA transposons and LTR retrotransposons than that of cyclic parthenogens of the same species (Rho *et al.* 2010; Schaack *et al.* 2010). Along a different line of evidence, a screen for widespread TE types in the putatively ancient asexual bdelloid rotifers failed to find *gypsy* and LINE elements, perhaps suggesting that their absence allowed bdelloids to persist as asexuals for millions of years (Arkhipova & Meselson 2000). However, subsequent work has revealed that bdelloids harbour a considerable diversity of TE types (reviewed in Gladyshev & Arkhipova 2010).

The parasitoid wasp *Leptopilina clavipes* is an ideal model system to study TE dynamics in asexuals. It occurs in both haplodiploid sexual (arrhenotokous) and asexual (thelytokous) populations, which are geographically separated. Northern European populations of this species have diverged from a Spanish population about 12 000–43 000 generations ago and have since become infected with parthenogenesis-inducing *Wolbachia* bacteria (Kraaijeveld *et al.* 2011). The *Wolbachia* has infected multiple females, and the northern populations of *L. clavipes* now consist of a series of genetically distinct clones (Kraaijeveld *et al.* 2011). Asexuality is achieved through gamete duplication by failure of chromosome segregation during the first mitotic division after meiosis (Pannebakker *et al.* 2004). This results in diploid, homozygous zygotes that develop as females. As meiosis proceeds normally, all TE-controlling mechanisms that are specific to meiosis are expected to be unaffected. However, it has been suggested that *Wolbachia* may affect the methylation state of the host genome (Negri *et al.* 2009). If *Wolbachia* removes methylation marks in a non-specific manner, this could potentially demethylate and reactivate silenced TEs.

We performed the first genome-wide comparison of copy number for all TEs simultaneously between closely related sexual and asexual lineages. We sequenced the entire genomes of an asexual and a sexual lineage of *L. clavipes* and assessed whether the asexual lineage has accumulated TE copies as predicted by theory. We estimated TE copy abundance from coverage depth (the number of sequence reads mapping to a particular reference sequence). Previous studies have demonstrated this to be an accurate approach (Alkan *et al.* 2009; Tenaillon *et al.* 2011). The most divergent TE in terms of copy number, a *gypsy*-like element, was selected for further investigation. For this element, we determined whether it was a component of the *Wolbachia* genome, whether genetically distinct asexual lineages had comparable TE loads and whether it was differentially methylated in asexual and sexual lineages.

Materials and methods

Wasp strains and Wolbachia *removal*

Asexual and sexual of *L. clavipes* were collected and cultured as described in Kraaijeveld *et al.* 2009;. The removal of *Wolbachia* using antibiotics is described in Kraaijeveld *et al.* 2009.

Whole-genome shotgun sequencing and assembly

Genomic DNA was extracted from a pool of 10 female wasps for each lineage. The genomes of the asexual females were completely homozygous because of *Wolbachia*-induced gamete duplication (Pannebakker *et al.* 2004), while the within-lineage genetic variability of the sexual lineage was severely reduced through approximately 50 generations of inbreeding in the laboratory (Kraaijeveld *et al.* 2011).

Both samples were sequenced three times on an Illumina GAIIx (two single-end runs and one paired-end run each, all 75 cycles). The entire data analysis was conducted independently for the two lineages. The sequences for the three runs were combined per lineage, and each was assembled de novo using SOAPdenovo (Li *et al.* 2010) (k-mer = 31). The contigs were screened for TEs using RepeatMasker (Smit *et al.* 2010), using the arthropod repeat library (Repbase 10; http://www.girinst.org/repbase/).

We estimated between-lineage difference in copy number for each TE-containing contig by calculating

read coverage depth. Estimating true copy number and identifying insertion sites was problematic because of the fragmentary nature of the genome assemblies. TE-containing contigs were extracted from the list of contigs. Most TE sequences identified by RepeatMasker covered the entire contig. For example, only 1.2% of the TE-containing contigs from the asexual assembly contained >1000 bp not covered by any particular TE. Furthermore, 28.1% of these contigs contained multiple TE-like sequences. Therefore, we mapped the original reads from the two single-end Illumina GAIIx runs for each lineage to the complete TE-containing contigs. We allowed for three mismatches in the seed to account for the possibility that homologous TEs showed small sequence differences between lineages. Coverage depth was calculated using Bedtools (Quinlan & Hall 2010). The coverage of TE-containing contigs by reads from the asexual and sexual lineages was tightly correlated for both genome assemblies (Pearson's correlation $r_{asexual} = 0.98$, $r_{sexual} = 0.99$). We assumed the deviation in slope from one to be due to slight differences between lineages in read number and read mappability. We therefore corrected for this difference in further analysis. We then calculated the fold difference between the coverages of the two lineages of the same contigs. This fold difference was log10-transformed and tested against the null expectation of mean = 0 using a one-sample *t*-test.

Identification of a gypsy*-like element and* forkhead *control gene*

We selected the longest contig from the cluster of contigs that showed the largest coverage differences between the asexual and sexual lineages for further analysis. We identified homologous sequences in the *Nasonia vitripennis* and *Bombyx mori* genomes using BLAST, which turned out to be the gag-pol polyprotein domain of retrotransposons from the *gypsy-Ty3* superfamily (GenBank accession numbers XM_001601092.1 and AB032718.1, respectively). To obtain a longer sequence, we designed degenerate primers (5′-AARYTNTAYGCNGCNAAY-3′ and 5′-RTARAARTTNACCATNCC-3′) that spanned 1110 bp of a reverse transcriptase domain within these homologous sequences. This fragment was resequenced in four *L. clavipes* lineages (two asexual, two sexual) to identify potential differences between lineages.

We searched for sequences homologous to the *forkhead* transcription factor gene, a putatively single-copy gene in the *Nasonia* genome, to be used as a control gene in qPCR. We discovered a 137 626-bp contig that showed significant similarity to predicted *forkhead*-like mRNAs in *Bombus terrestris* and *Apis mellifera* (Genbank accession numbers XM_003397859.1 and XM_394770.4, respectively) in a BLAST search. Species-specific primers were developed (5′-GGATGCAGAGTCCAGAAGGA-3′ and 5′-TTGGCAAAATTCCATTAGGC-3′) that amplified a 105-bp region, and these were used in qPCR.

Quantitative PCR

We designed a set of primers spanning a 109-bp region in the reverse transcriptase domain of the *gypsy*-like element (5′-CGTTCGGTCTGTTCGAATTT-3′ and 5′-GCG AAACAGAAGTCCAATCC-3′). Genomic DNA was extracted from one recently emerged female per lineage (Qiagen blood and tissue kit). Relative copy numbers of the *gypsy*-like element and *forkhead* sequences were quantified using the Lightcycler LC-480 qPCR system (Roche). The qPCRs contained 5 μL qPCR MasterMix for SYBR Green (Bio-Rad), 60 ng DNA and 300 nM of each primer and were run in duplicates using the following two-step cycling programme: 95 °C for 15 s, 60 °C for 1 min for 40 cycles. Efficiency of the PCR was estimated from the samples separately for the two genes using the software LinRegPCR. Data from qPCR were analysed using the second derivative maximum method. Here, the Cp-value represents the cycle at which the increase in fluorescence is highest and where the logarithmic phase of a PCR begins. The mean Cp of the duplicates for the TE was calibrated on one of the sexual samples (PCR efficiency dCp) and then normalized on the control gene.

Southern blot analysis

Genomic DNA was extracted from 10 recently emerged females per lineage using phenol/chloroform. Ten microgram of DNA per lineage was digested with *Eco*RI and electrophoresed on a 0.7% agarose gel. The DNA was blotted onto hybond+ membrane (Amersham Biosciences), which was then probed with the DIG-labelled 1110 bp gypsy-like sequence described above. Hybridization and detection were performed using the DIG labelling protocol (Roche Applied Sciences). The intensity of each band was quantified using IMAGEJ, normalized on the intensity of the marker band and corrected for the amount of input DNA based on the intensity of smears on the ethidium bromide-stained gel. The relative intensities were ln-transformed and compared using ANOVA.

Bisulphite sequencing

For the *gypsy*-like element, bisulphite-treated samples were PCR amplified with primers 5′-GGTAAATG-AAGAAATGYYAAGTAT-3′ and 5′-CAATCACCRAA-

AATATTRTTTTTCC-3′. The PCR product was cleaned and sequenced following standard procedures. Bisulphite sequencing data were analysed using Kismeth (Gruntman *et al.* 2008).

Results

Genome-wide TE copy number

To compare genome-wide TE loads between asexual and sexual *L. clavipes,* we applied the following approach. (i) We sequenced the genomes of an asexual lineage and a sexual lineage and assembled these *de novo* independently; (ii) We screened both genome assemblies for TE-like sequences using RepeatMasker; and (iii) We assessed copy number for each repeat-containing contig by mapping the original reads to these contigs and calculating coverage. TEs represented by many copies should be more highly covered than TEs with few copies.

The Illumina GAIIx sequencing resulted in 5.86 and 4.76 Gb of sequence for the asexual and sexual lineages, respectively. Assuming a genome size comparable to that of *Nasonia* (300 Mb), this covered the genome approximately 19.5 and 15.9 times, respectively. One hundred and eighty-eight and 98 Mb were assembled *de novo* into contigs >100 bp for the asexual and sexual lineages, respectively (Table 1). Combining all data for the two lineages into a single analysis did not significantly improve the assembly (data not shown). Given that the latter approach could have resulted in chimeric contigs, we opted to assemble the two genomes separately.

RepeatMasker identified 3.2 and 2.4 Mb of TE sequence in the asexual and sexual genome assemblies, respectively. Most major TE classes (DNA transposons, LTR and LINE-like retrotransposons) were represented. DNA transposons and LTR retrotransposons were particularly numerous. However, the number of individual elements identified by RepeatMasker may be an unreliable estimate of true copy number as it depends critically on the quality of the genome assembly. For example, identical TE copies are likely to be collapsed into one contig.

Table 1 Details of the de novo genome assemblies for the sexual and asexual *Leptopilina clavipes* lineages

	Asexual	Sexual
k-mer length	31	31
Contigs > 100 bp	659	423 887
Total bp in contigs > 100 bp	187 960 762	97 695 769
Contig n50	379	259
Average contig coverage	110	158
Scaffolds	158 271	8856
Total bp in scaffolds	147 170 984	5 669 804
Scaffold n50	873	260

To avoid bias resulting from differences in contig length, we mapped the reads from both the asexual and sexual lineages to the asexual and sexual genome assemblies. This resulted in four sets of coverage estimates, which we compared within genome assembly. The mean contig coverage was 4% higher for the asexual lineage than for the sexual lineage in the asexual genome assembly. For the sexual genome assembly, this difference was 6%. We corrected for these differences in further analysis by increasing the coverage estimates for the sexual lineage by 4% and 6%, respectively. Coverage of TE-containing contigs was higher for the asexual lineage than for the sexual lineage for both genome assemblies, although the mean difference was very small in both cases and only marginally significant for the sexual assembly (Fig. 1; asexual assembly: $t_{10730} = 9.41$, $P < 2.2e\text{-}16$, sexual assembly: $t_{7691} = 2.48$, $P = 0.013$). For both genome assemblies, DNA transposons were more highly covered by the asexual lineage compared to the sexual lineage (Table 2, Fig. 2). By contrast, LINE and LTR elements showed only marginal differences in mean coverage between the lineages (Table 2). For LTR elements, the difference was only significant for one of the assemblies (Table 2). The repeat-containing contigs from the sexual lineage showed a cluster of contigs for which asexual lineage yielded three to five times higher coverage than the sexual lineage (bottom-right in Fig. 1B). These contigs were not assembled for the asexual lineage. These outliers consisted of five *gypsy*-like sequences and two *Pao*-like LTR sequences. It is likely that these seven contigs represent fewer actual TEs, as two pairs of contigs aligned to the same TE when aligned to the *Nasonia vitripennis* genome. No such outliers were detected among the contigs from the asexual lineage (Fig. 1A).

Copy number of gypsy*-like element*

The cluster of seven small (<500 bp) contigs that showed the most dramatic difference in coverage between the asexual and sexual lineages (bottom-right in Fig. 1B) was dominated by sequences resembling *gypsy* retrotransposable elements. We further characterized 1110 bp of one of these elements using degenerate PCR. The element was Sanger sequenced in four *L. clavipes* lineages (two asexual and two sexual), and the sequences were identical to each other.

The number of Illumina reads from the asexual lineage aligning to this extended *gypsy*-like sequence was 4.3 times as high as for the sexual lineage (corrected for the difference in total read number). The number of reads per base from the sexual lineage aligning to the

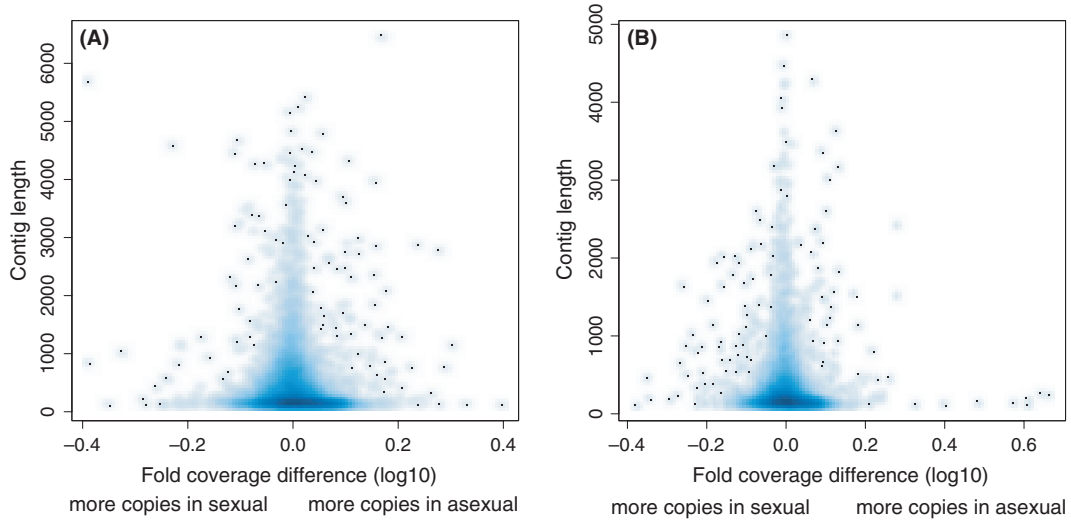

Fig. 1 Comparison of coverage depth for all transposable elements (TEs) between the sexual and asexual lineages. Scatterplot of the fold difference (log10-transformed) in coverage depth per TE-containing contig between asexual and sexual lineages, plotted against contig length. The density of data points is indicated in blue and was obtained using a kernel density estimate. (A) Contigs from the genome assembly of the asexual lineage; (B) contigs from the genome assembly of the sexual lineage. Both plots are corrected for the difference in read number between the two lineages and are thus centred on 0. Note that the density of data points extends towards the right in both figures, indicating relatively large numbers of contigs that are more deeply covered by the reads from the asexual lineage.

Table 2 Results of single-sample *t*-tests of coverage differences between asexual and sexual lineages for the major transposon types, tested against the null expectation of no difference. *N* equals the number of sequences for that transposable element type among the contigs

	Asexual assembly			Sexual assembly		
	n	*t*	*P*	*n*	*t*	*P*
DNA transposons	5618	9.51	0.0000	3638	9.62	0.0000
LTR	3892	−0.53	0.5980	3403	−3.27	0.0011
LINE	763	−3.77	0.0002	525	−2.85	0.0045

gypsy-like element was two times higher than to the putatively single-copy gene *forkhead*. This indicates that the initial copy number for this element was two and has increased to nine in the asexual lineage. We validated this result using quantitative real-time PCR (qPCR) and Southern blotting. We quantified the relative copy number of this TE in the genomic DNA of females from 12 asexual and 9 sexual lineages of *L. clavipes*. The 12 asexual lineages represent eight of the 11 genetically distinct asexual lineages, and the nine sexual lineages represented nine genotypes (Kraaijeveld *et al.* 2011). We performed qPCR of a 109-bp fragment of the reverse transcriptase domain of the *gypsy*-like element, using the *forkhead* transcription factor as a control. qPCR revealed that, without exception, the asexual lineages had higher copy numbers than the sexual lineages, although there was some variation between lineages. The average fold difference in copy number relative to the control gene between asexual and sexual lineages was 4.1 (Fig. 3A; $\text{mean}_{\text{asexual}} = 4.22$, $n_{\text{asexual}} = 10$, $\text{mean}_{\text{sexual}} = 1.03$ $n_{\text{sexual}} = 9$, Wilcoxon $W = 90$, $P < 0.00001$), very similar to the difference found using Illumina read coverage. Southern blot analysis also confirmed this result (Fig. 3B). Probing *Eco*RI-digested DNA with the known sequence of the *gypsy*-like element produced a single band at approximately 700 bp, indicating that the full element has an additional *Eco*RI restriction site outside the known sequence and that the probe used detected an internal fragment of the *gypsy*-like TE. This band was on average 3.5 times more intense in the asexual lineages ($n = 12$) compared to the sexual lineages ($n = 8$; corrected for the amount of input DNA (Fig. S1, Supporting information); $F_{1,18} = 234.32$, $P < 0.0000001$; Fig. 2B).

The between-lineage differences in copy number of the *gypsy*-like element (qPCR results) were correlated negatively to the pairwise genetic relationships between these lineages (microsatellite data; Kraaijevel *et al.* 2011; Mantel test: $r_{\text{asexual}} = -0.38$, $P_{\text{asexual}} = 0.05$, $r_{\text{sexual}} = -0.39$, $P_{\text{sexual}} = 0.02$), indicating that closely related lineages resembled each other in copy number of the *gypsy*-like element. The asexual lineages have thus accumulated copies of this element independently.

To investigate whether this difference was because of this element being present in the *Wolbachia* genome, we

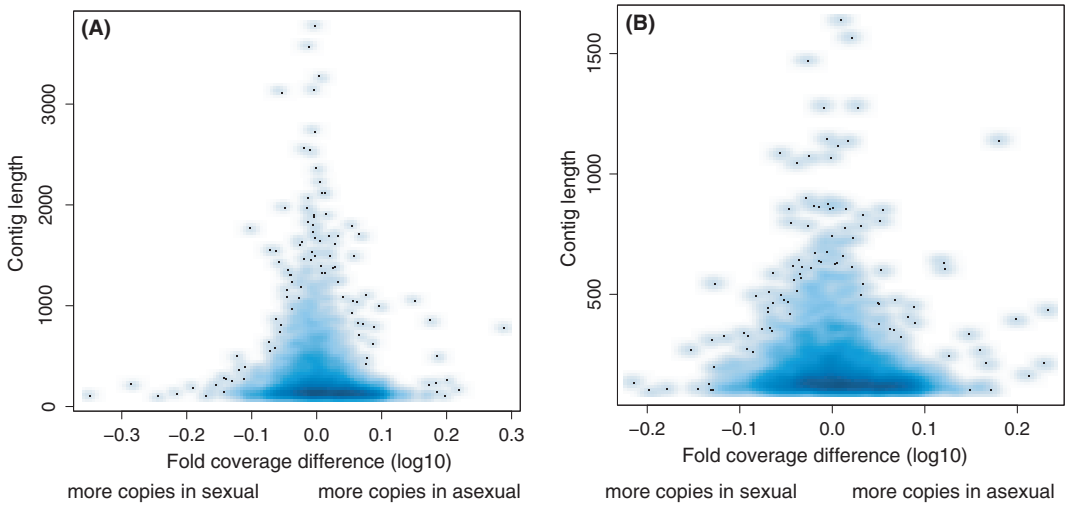

Fig. 2 The asexual lineage harbours more copies of DNA transposons than the sexual lineage. Scatterplot of the fold difference (log10-transformed) in coverage depth per DNA transposon-containing contig for asexual and sexual lineages, plotted against contig length. (A) Contigs from the genome assembly of the asexual lineage; (B) contigs from the genome assembly of the sexual lineage. For details, see Fig. 1.

repeated the qPCR analysis using asexual females from which *Wolbachia* had been removed using antibiotics. The difference in copy number relative to the control gene remained significant and of the same magnitude (Fig. 3A; $\text{mean}_{\text{asexual}} = 4.17$, $n_{\text{asexual}} = 10$, $\text{mean}_{\text{sexual}} = 1.06$, $n_{\text{sexual}} = 9$, Wilcoxon $W = 90$, $P = 0.00016$). The Cp ratios of the antibiotic-treated samples were highly correlated to those of the non-treated samples ($r = 0.84$, $P < 0.0001$).

Figure 4 shows the depth of coverage across the 1110 bp of known sequence of the *gypsy*-like TE for the asexual and sexual lineage. While the coverage is very different between these lineages, the pattern of coverage across the sequence is similar, indicating that at least this part of the element has transposed intact.

Transposable element methylation

BLAST searches identified two contigs in the genome assembly of the asexual lineages showing strong similarity to the methylation gene *dmnt1* from *Nasonia*, indicating that *L. clavipes* is capable of DNA methylation. To test whether the increased TE copy numbers are the result of *Wolbachia*-induced demethylation, we measured DNA methylation using bisulphite sequencing.

A 373-bp portion of the *gypsy*-like element was bisulphite sequenced in eight samples (two lineages each for asexual, asexual antibiotic-treated, sexual and sexual antibiotic-treated). The results showed that <1% of cytosines were methylated in any sequence (Fig. S2, Supporting information), which did not exceed the background rate of false positives. Thus, there was no evidence that the *gypsy*-like element in *L. clavipes* is methylated in either asexual or sexual strains, excluding *Wolbachia*-induced demethylation as an explanation for the difference in copy number of the *gypsy*-like TE between asexual and sexual lineages.

Discussion

Our results showed that *L. clavipes*, like the model parasitoid *Nasonia* (Werren *et al*. 2010), harbours a diverse array of TEs that include DNA transposons, LTR and LINE-like elements. The asexual lineage of *L. clavipes* had higher copy numbers of DNA transposons than the sexual lineage, while the average between-lineage differences in LTR- and LINE-like retrotransposable elements were marginal. However, one or a small number of *gypsy*-like elements showed a fourfold higher copy number in the asexual lineage compared to the sexual lineage, which was consistent between lineages. These patterns suggest that *Wolbachia*-induced parthenogenesis has had different effects on different types of TE.

The relative abundance of DNA transposon copies in the asexual lineage is at odds with the pattern observed in the water flea *D. pulex*. In that species, obligate parthenogens appear to have lower copy numbers for several families of DNA transposons than populations that have regular episodes of sexual reproduction (Valizadeh & Crease 2008; Schaack *et al*. 2010). The difference with our results may be due to the different cytological mechanisms involved in achieving parthenogenesis (Fig. 5). The production of parthenogenetic eggs in *D. pulex* appears to involve a failure of chromosome

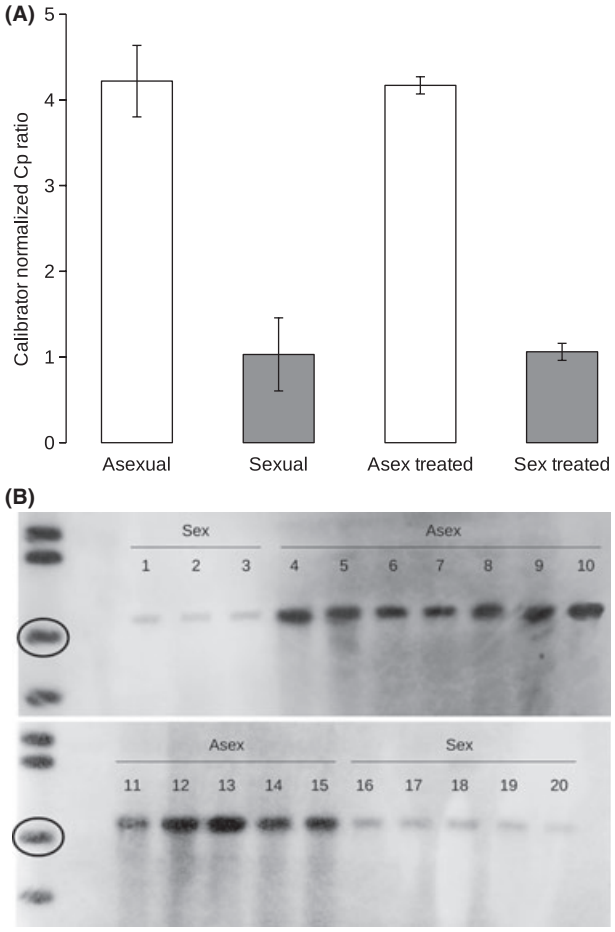

Fig. 3 A *gypsy*-like retrotransposon has a fourfold higher copy number in the asexual lineage compared to the sexual lineage. (A) Quantitative PCR results: calibrated normalized Cp_{gypsy}/Cp_{fkh} ratios for 10 asexual and 9 sexual lineages (untreated) and 9 asexual and 9 sexual lineages from which *Wolbachia* had been removed using antibiotics (mean ± SE). (B) Southern blot analysis of genomic DNA digested with *Eco*RI and probed with the gypsy-like sequence. The first lane contains the size marker (*Hin*dIII-digested lambda). Each sample lane contains DNA extracted from 10 female wasps from a single lineage. The marker band used for normalizing the intensities is circled.

segregation at the first meiotic anaphase, which renders the resulting offspring heterozygous (Hiruta *et al.* 2010). By contrast, the production of parthenogenetic eggs in *L. clavipes* involves the failure of chromosome segregation during the first mitotic division after meiosis, which results in completely homozygous offspring (Pannebakker *et al.* 2004). Homozygosity reduces the chance of future excision of DNA transposons, because an excised copy can be reconstituted when the excision site is repaired using the homologous chromosome carrying a non-excised copy as template. Consequently, parthenogenetic populations of *D. pulex* may lose heterozygous TE copies, while those of *L. clavipes* may not. Furthermore, the cut-and-paste mechanism of DNA transposons effectively changes to a copy-and-paste mechanism under the above scenario, which would lead to faster accumulation of DNA transposon copies in *L. clavipes* compared to *D. pulex*.

We found no consistent differences in copy number of LTR- and LINE-like TEs between asexual and sexual lineages. However, one or several *gypsy*-like TEs have accumulated many copies in the asexual lineages because the latter split from the ancestor that it shared with the sexual lineage. Again, these results are inconsistent with published results for *D. pulex*, in which one of two analysed LTR families showed higher copy numbers in cyclic parthenogens compared to obligate parthenogens (Schaack *et al.* 2010). However, whether this is representative for the other retroelements in the genome of *D. pulex* is unknown. The two lineages of *L. clavipes* differ in several respects, which may provide different explanations for the difference in *gypsy* copy number. First, a population bottleneck may have occurred in the sexual ancestor of the asexual populations, but not in the ancestor of the extant sexual populations. If so, this could have increased TE copy number through genetic drift. However, the northern populations display considerable clonal genetic diversity, which is a remnant of the genetic variation that existed in the ancestral arrhenotokous population (Kraaijeveld *et al.* 2011). This indicates that the ancestral population did not experience significantly reduced effective population size. Furthermore, a population bottleneck would be expected to affect many TEs, rather than one or a few specific ones. Second, the asexual lineages are infected with *Wolbachia*, while the sexual lineages are not. The difference in copy number for the *gypsy*-like element persisted when *Wolbachia* was removed using antibiotics, indicating that this element is not located on the *Wolbachia* genome. Furthermore, this element was not differentially methylated in asexual and sexual lineages, excluding a role for *Wolbachia*-induced demethylation. However, the increased activity of the *gypsy*-like TE may have been a side effect of other manipulations

Fig. 4 Coverage across the 1110 bp of the *gypsy*-like transposable element for the asexual and sexual lineages (top and bottom panel, respectively). Note that the scale of the two panels differs.

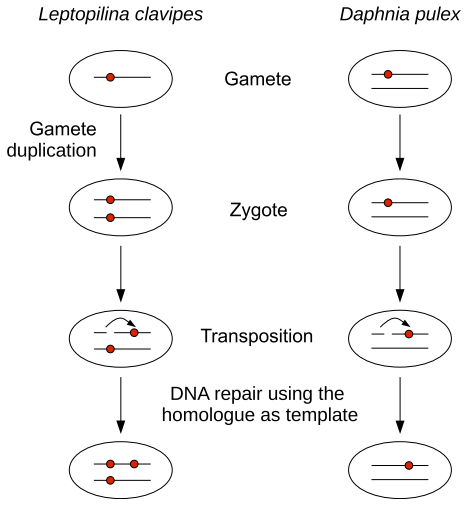

Fig. 5 Schematic representation of the argument put forward in the discussion explains the different patterns of DNA transposon abundance in *Leptopilina clavipes* and *Daphnia pulex*. The lines represent the host genome and the dots DNA transposons.

of the host genome by *Wolbachia*. For example, *Wolbachia*-induced gamete duplication could target proteins from the Argonaut family, which are involved in meiosis and oogenesis in many organisms (Thomson & Lin 2009). Proteins from the Argonaut family, such as Piwi, Aubergine and Ago3, are also involved in RNAi-mediated silencing of *gypsy* TEs in *Drosophila* (Brennecke *et al*. 2007). Thus, deregulation of Argonaut proteins by *Wolbachia* may interfere with TE silencing and potentially reactivate TEs. Alternatively, the element may have been active in both lineages, but the asexual lineage may have been less able to prevent the accumulation of new copies because of the inefficiency of natural selection against highly loaded genotypes in the absence of sexual recombination or of failing TE suppression mechanism(s).

All asexual lineages harboured high copy numbers of the *gypsy*-like element, and the differences in copy numbers between asexual lineages were correlated to the genetic distances between them. If the accumulation of copies of this element was indeed linked to the switch to asexuality as suggested above, then it happened in all asexual lineages. Subsequent transposition rates must have been fairly uniform across lineages to result in similar copy numbers. Alternatively, the consistency between lineages may indicate a ceiling beyond which transposition becomes lethal or epigenetically repressed.

No signs of fitness reduction in asexual lineages of *L. clavipes* have so far been detected in the traits assayed (brood size: Pannebakker *et al*. 2005; longevity: Reumer *et al*. 2007), although traits involved in sexual reproduction have clearly decayed (Pannebakker *et al*. 2005; Kraaijeveld *et al*. 2009). However, if the asexual lineages continue to gain TE copies, negative fitness effects would be expected in the future.

Our study shows that the switch to asexuality in *L. clavipes* was indeed accompanied by the proliferation of certain types of TEs. However, the cause of this accumulation of TEs was probably not inefficient natural selection in asexuals, as originally envisioned. Instead, we suggest that the specific cytological mechanism that causes asexuality may cause proliferation of specific transposons as a side effect. Asexual reproduction is achieved in a myriad of ways in nature, many of which may cause proliferation of particular TEs.

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K.K's research focusses on the infection dynamics of parthenogenesis-inducing *Wolbachia* and their consequences for host evolution. For BZ, this paper is part of her MSc research. B.H. works on the analysis of mutations and epigenetics regulation leading to disease. C.V. is professor in evolutionary biology and focusses on transposable element dynamics. S.d.P.'s research involves DNA repair and development in plants. J.A. uses molecular tools to study population structure in amphibians and insect parasitoids. J.d.D is professor in medical genome technology and focuses on the use of new high-throughput genome technology in medical and biological research. P.K. is a professor of human genetics, specializing in population genetics.

Data accessibility

Fastq files containing the Illumina sequence reads used for this study and the associated analysis scripts are available at Dryad: doi: 10.5061/dryad.k3bh40bg. DNA sequence for the *gypsy*-like TE: GenBank accession number, HM999657.1.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Ethidium bromide-stained agarose gel used for the southern blot depicted in Fig. 3b.

Fig. S2 Cytosine methylation patterns of the gypsy-like transposable element of *Leptopilina clavipes*.

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