Transposable element proliferation as a possible side effect of endosymbiont manipulations

Ken Kraaijeveld[1,*] and Jens Bast[2]

[1]Leiden Genome Technology Center; Leiden University Medical Center; Leiden, The Netherlands; [2]J.F. Blumenbach Institute of Zoology and Anthropology; University of Goettingen; Goettingen, Germany

*Correspondence to: Ken Kraaijeveld;
Email: ken@kenkraaijeveld.nl

The mode of reproduction has been predicted to affect the proliferation of transposable elements (TEs). A population that switches from sexual to asexual reproduction could either accumulate TEs because purifying selection becomes less efficient, or a decrease in TE load because the opportunity for horizontal transmission is reduced. A third possibility is that the mechanism that induces asexual reproduction affects TE dynamics as a side effect. We propose two such mechanisms that might explain recently described patterns of TE abundance in sexual and asexual lineages of the parasitoid wasp *Leptopilina clavipes*. Asexual reproduction in this species is induced by endosymbiotic *Wolbachia* bacteria. In order to achieve parthenogenesis in its host, *Wolbachia* might remove methylation or interfere with Argonaute proteins. Both methylation and Argonaute proteins are known to control TE activity in other species. By interfering with either, *Wolbachia* might therefore secondarily hamper the control of specific TEs.

The relationship between mode of reproduction and transposable element (TE) dynamics has been the topic of considerable debate (summarized in ref. 1). On the one hand, purifying selection is expected to be less efficient in asexual compared with sexual taxa, leading to an accumulation of TE copies in asexuals. On the other hand, sex allows horizontal transmission of TEs and will facilitate the spread of TEs. Which of these driving factors, if any, will be most important is currently an unresolved question. Recent advances in DNA sequencing technology now allow us to address this question on a genome-wide scale. In a recent paper published in *Molecular Ecology*, we quantified TE loads in sexual and asexual lineages of the parasitoid wasp *Leptopilina clavipes*.[2] Parthenogenesis in this species is induced by endosymbiotic *Wolbachia* bacteria, that are thought to have infected *L. clavipes* several thousand years ago.[3] Uninfected lineages reproduce sexually. The results of our study were inconsistent with models that predict increases[4] or decreases[5] in TE load in asexuals compared with sexuals, regardless of TE type. Instead, we found markedly different patterns between the various types of TEs. Loads of DNA transposons were higher in asexuals, while there was no difference between sexuals and asexuals for LTR and LINE-like TEs, except for one or a few *gypsy*-like LTR elements. The reasons for these patterns have already been the subject of some speculation.[1,2] Here, we elaborate on the possibility that TE dynamics are affected by *Wolbachia*. More precisely, we suggest that in order to induce parthenogenesis, *Wolbachia* has to interfere with host cellular processes, which secondarily also interferes with the control of TE activity. While these suggestions are purely speculative at this moment, we discuss them here because we believe that such processes could be of widespread importance.

We suggest two ways in which *Wolbachia*-induced manipulation of the host reproductive machinery could interfere with the repression of particular TE
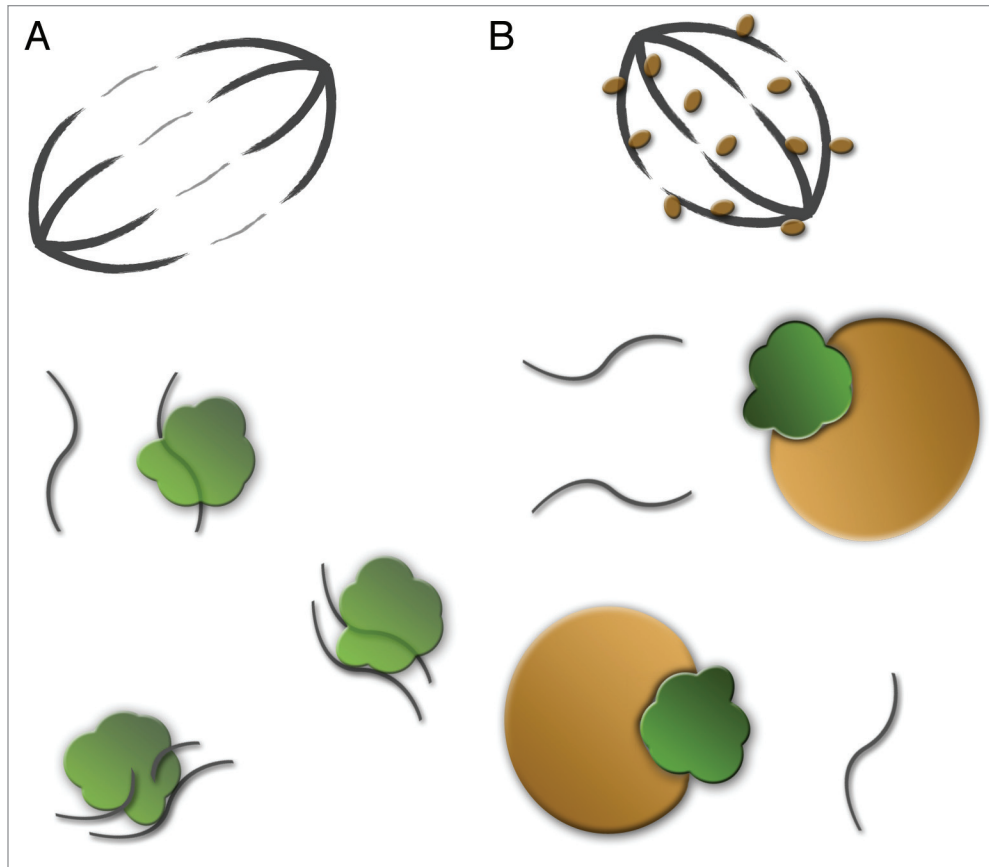
Figure 1. (**A**) Cartoon of a normal dividing cell with meotic/mitotic spindle at the top. Complexes of Argonaute proteins (green) and antisense TE fragments capture and destroy TE mRNAs. (**B**) Dividing cell infected with *Wolbachia*. *Wolbachia* (brown) associate with microtubuli (top) and capture Argonaute proteins (green). TE derived mRNAs are left to insert back into the host genome.

types. These mechanisms are illustrated in **Figures 1 and 2**.

First, *Wolbachia* might interfere with the normal functioning of proteins from the Argonaute family (**Fig. 1**). Argonaute-like proteins are involved in many cellular processes, including cell division and gametogenesis.[6] In order for *Wolbachia* to make unfertilized *L. clavipes* eggs develop as females, it has to ensure that these become diploid. It does so by preventing chromosome segregation at the first mitotic division after meiosis.[7] Thus, in gametes infected by *Wolbachia*, the chromosomes duplicate, condense, but then enter G1 without completing mitosis or cytokinesis. The molecular mechanism through which *Wolbachia* achieves this effect is currently unknown. However, one way for *Wolbachia* to prevent the chromosomes from separating after duplication might be to interfere with Argonaute proteins. In mice for example, mutants defective for a protein from the

Argonaute family show arrest during early meiosis.[8]

In addition to their role in cell cycle regulation, Argonaute proteins play an important role in the control of TE activity, through a mechanism known as the ping-pong model.[9,10] Briefly, Argonaute proteins form a complex with short antisense sequences transcribed from defective TEs. These target full-length TE transcripts (**Fig. 1A**), which they then degrade, resulting in new short sense TE fragments that can bind to other Argonaute proteins. These in turn target antisense transcripts from the defective TEs, resulting in more antisense bait, and so on. If *Wolbachia* would interfere with the abundance or functioning of Argonaute proteins as suggested above, it would automatically hamper the Argonaute-driven capturing and degradation of TE mRNA (**Fig. 1B**). These TE transcripts are then left free to be reverse transcribed into cDNA and pasted back into the genome.

A second way in which *Wolbachia*-induced manipulation of the host could lead to proliferation of TEs is by disturbing normal patterns of DNA methylation. To make unfertilized eggs develop as females, it is not enough for *Wolbachia* to cause diplodization of the gametes as described above. *Wolbachia* also has to prevent diploid zygotes from developing as diploid males. To do so, *Wolbachia* has to manipulate the host's sex determination mechanism. Several sex determination mechanisms are known in hymenoptera[11] and it is currently unknown which of these applies to *L. clavipes*. However, since strong inbreeding does not result in diploid males in *L. clavipes* (Kraaijeveld, personal observation), sex determination is unlikely to be based on allelic differences at one or a few genetic loci as in for example the honey bee *Apis melifera*. We therefore assume that sex determination in *L. clavipes* is most likely similar to that described for another parasitoid

wasp, *Nasonia vitripennis*. In *Nasonia*, female development requires at least one active copy of the gene *transformer* (*tra*) or a trans-acting factor that regulates *tra* expression.[12] *Tra* is silenced in the female germline, so simple gamete duplication would result in two silenced copies of *tra* and hence male development. In the male germline, however, *tra* is not silenced and males transfer an active copy of *tra* to their offspring. Fertilized offspring therefore inherit both an active and a silenced copy of tra and develop as females. To achieve female development of diploidized zygotes, *Wolbachia* has to emulate the male germline and remove silencing of *tra*. The mechanism through which *tra* is silenced is not known, but may involve DNA methylation. If so, *Wolbachia* could either demethylate the host genome completely, or remove methylation only from *tra*. In the latter case, demethylation could spread to nearby regions of the genome, analogous to the spread of methylation from silenced TEs to nearby genes that has been observed in several species.[13-15]

Methylation is a common way of silencing TEs. For example, mutant *Arabidopsis* plants that are defective in their methylation machinery experience bursts of TEs that are normally silent.[16] If *Wolbachia* removes methylation marks in a non-specific manner to induce female development of the zygote, it may also demethylate nearby TEs, thereby reactivating them (**Fig. 2**).

Whether either of the above mechanisms actually operates in the *L. clavipes-Wolbachia* system is at this stage unknown. We made a start testing the methylation hypothesis by checking for methylation of *gypsy* in sexual and asexual *L. clavipes*. We found that *gypsy* was not methylated in either,[2] suggesting that hypothesis 2 cannot account for the high copy number of *gypsy* in asexual *L. clavipes*. We have not tested the first hypothesis. Our reason for elaborating on the ideas here is because mechanisms like these could play an important role in many systems. Molecular mechanisms that control TE proliferation are often closely related to other important processes. As we point out here, methylation controls the transcription of host genes and TEs. Likewise, the ping-pong model for controlling TEs
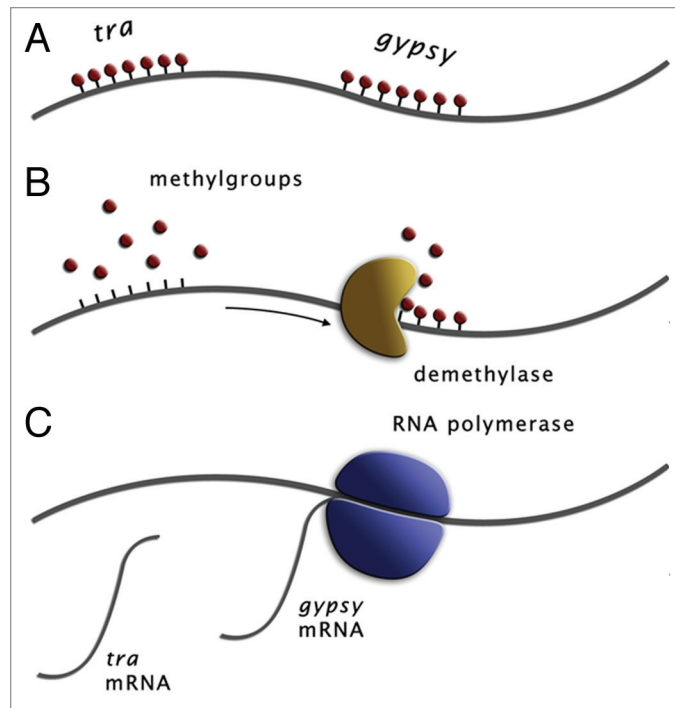
Figure 2. Cartoon of chromosomal region in female germline. (**A**) The sex determination gene *tra* is methylated as well as a nearby *gypsy* element. (**B**) In order to induce female development, *Wolbachia* produces a demethylase that removes methyl groups. (**C**) In the absence of methylation, both *tra* and *gypsy* can be transcribed.

post-transcriptionally contains components that are important in a wide variety of cellular processes. Other mechanisms that control TEs may similarly have other functions in host cells. It follows that interference of methylation, Argonaute proteins or other mechanisms by endosymbiotic bacteria or other environmental factors would disrupt multiple processes at once, including TE control.

Acknowledgments

References

1. Crespi B, Schwander T. Asexual evolution: do intragenomic parasites maintain sex? Mol Ecol 2012; 21:3893-5; PMID:22835046; http://dx.doi.org/10.1111/j.1365-294X.2012.05638.x.
2. Kraaijeveld K, Zwanenburg B, Hubert B, Vieira C, De Pater S, Van Alphen JJM, et al. Transposon proliferation in an asexual parasitoid. Mol Ecol 2012; 21:3898-906; PMID:22548357; http://dx.doi.org/10.1111/j.1365-294X.2012.5582.x.
3. Kraaijeveld K, Franco P, de Knijff P, Stouthamer R, van Alphen JJM. Clonal genetic variation in a Wolbachia-infected asexual wasp: horizontal transmission or historical sex? Mol Ecol 2011; 20:3644-52; PMID:21668807.
4. Dolgin ES, Charlesworth B. The fate of transposable elements in asexual populations. Genetics 2006; 174:817-27; PMID:16888330; http://dx.doi.org/10.1534/genetics.106.060434.
5. Wright S, Finnegan D. Genome evolution: sex and the transposable element. Curr Biol 2001; 11:R296-9; PMID:11369217; http://dx.doi.org/10.1016/S0960-9822(01)00168-3.
6. Thomson T, Lin H. The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. Annu Rev Cell Dev Biol 2009; 25:355-76; PMID:19575643; http://dx.doi.org/10.1146/annurev.cellbio.24.110707.175327.
7. Pannebakker BA, Pijnacker LP, Zwaan BJ, Beukeboom LW. Cytology of Wolbachia-induced parthenogenesis in Leptopilina clavipes (Hymenoptera: Figitidae). Genome 2004; 47:299-303; PMID:15060582; http://dx.doi.org/10.1139/g03-137.
8. Carmell MA, Girard A, van de Kant HJ, Bourc'his D, Bestor TH, de Rooij DG, et al. MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. Dev Cell 2007; 12:503-14; PMID:17395546; http://dx.doi.org/10.1016/j.devcel.2007.03.001.
9. Aravin AA, Hannon GJ, Brennecke J. The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. Science 2007; 318:761-4; PMID:17975059; http://dx.doi.org/10.1126/science.1146484.
10. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, et al. Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell 2007; 128:1089-103; PMID:17346786; http://dx.doi.org/10.1016/j.cell.2007.01.043.
11. Sánchez L. Sex-determining mechanisms in insects. Int J Dev Biol 2008; 52:837-56; PMID:18956315; http://dx.doi.org/10.1387/ijdb.072396ls.

12. Verhulst EC, Beukeboom LW, van de Zande L. Maternal control of haplodiploid sex determination in the wasp Nasonia. Science 2010; 328:620-3; PMID:20431014; http://dx.doi.org/10.1126/science.1185805.

13. Kinoshita Y, Saze H, Kinoshita T, Miura A, Soppe WJ, Koornneef M, et al. Control of FWA gene silencing in Arabidopsis thaliana by SINE-related direct repeats. Plant J 2007; 49:38-45; PMID:17144899; http://dx.doi.org/10.1111/j.1365-313X.2006.02936.x.

14. Martin A, Troadec C, Boualem A, Rajab M, Fernandez R, Morin H, et al. A transposon-induced epigenetic change leads to sex determination in melon. Nature 2009; 461:1135-8; PMID:19847267; http://dx.doi.org/10.1038/nature08498.

15. Rebollo R, Karimi MM, Bilenky M, Gagnier L, Miceli-Royer K, Zhang Y, et al. Retrotransposon-induced heterochromatin spreading in the mouse revealed by insertional polymorphisms. PLoS Genet 2011; 7:e1002301; PMID:21980304; http://dx.doi.org/10.1371/journal.pgen.1002301.

16. Tsukahara S, Kobayashi A, Kawabe A, Mathieu O, Miura A, Kakutani T. Bursts of retrotransposition reproduced in Arabidopsis. Nature 2009; 461:423-6; PMID:19734880; http://dx.doi.org/10.1038/nature08351.