Male or female?
The epigenetic conflict between a feminizing bacterium and its insect host

Ilaria Negri,1,* Peter J. Mazzoglio,1 Antonella Franchini,2 Mauro Mandrioli2 and Alberto Alma1
1DIVAPRA—Entomology and Zoology Applied to the Environment; University of Turin; Grugliasco (Turin), Italy; 2Department of Animal Biology; University of Modena; Modena, Italy

In the leafhopper *Zyginidia pullula*, the maternally-inherited symbiont *Wolbachia pipientis* feminizes genetic males, leaving them as intersex females. The Wolbachia density is correlated with the feminization degree of males which either bear ovaries or testes. Methylation-sensitive RAPD profiles showed that while feminized males with ovaries possess a female imprinting pattern, those with testes maintain the same methylation pattern of males, indicating that the Wolbachia infection is able to modulate host genomic imprinting, but this occurs only if the bacterium exceeds a density threshold. Here we report methylation-sensitive RAPD analyses on gonads (testes and ovaries) from leafhopper uninfected male and female individuals, and males feminized by Wolbachia, discussing these additional insights and speculating on possible bacterium/host interactions.

The alfa-proteobacterium *Wolbachia pipientis* is a widespread maternally-inherited endosymbiont of arthropods, known to induce several reproductive manipulations in the host, such as cytoplasmic incompatibility between gametes, male killing, feminization and parthenogenesis.1 Even if Wolbachia bacteria are detected in somatic tissues such as muscles, brain, gut, salivary glands, fat body, Malpighian tubules and haemolymph, they primarily localize in the host’s gonads.2 Wolbachia are transmitted predominately through the female germ-line: during embryogenesis, in *Drosophila melanogaster* the bacteria get incorporated into the pole cells, the precursors of germ-line stem cells, or they may enter the germ-cell line through the somatic stem cell niches.3 This behavior makes Wolbachia an excellent candidate as an “environmental” factor promoting epigenetic changes in host gene expression, which may be inherited by future generations.

In the leafhopper *Zyginidia pullula* (Hemiptera, Cicadellidae) Wolbachia-infected males develop into neo-females or intersexes with a female phenotype, which retain a secondary male feature.4 The feminized males readily mate with uninfected males and can produce viable offspring.4 In a recent paper, epigenetic differences between *Z. pullula* males and females were detected with a Methylation-sensitive Random Amplified Polymorphic DNA (RAPD) approach that showed a sex-specific genome methylation pattern in *Z. pullula* somatic tissues.5 RAPD profiles showed that leafhopper males feminized by Wolbachia possess the same imprinting pattern as genetic females.5 Since Wolbachia are usually present at a higher density in host gonads, here we show methylation-sensitive RAPD profiles obtained with DNA extracted from gonads of *Z. pullula* adults (males, females and feminized males).

Genomic imprinting is a phenomenon whereby a gene, or a region of a chromosome, is reversibly modified so that it retains a sort of “memory” of its own genetic history. The term imprinting indicates a situation in which the activity of the imprinted genes or chromosomes is determined by the sex of the parent that transmits them and the altered expression
is limited to the somatic tissue of the progeny, whereas the germ-line is not permanently altered. Epigenetic changes are based on molecular processes that can activate, reduce or completely disable the activity of genes, and methylation of cytosine residues in the DNA is currently one of the most studied epigenetic mechanisms.

Until now, genomic imprinting has been found in many vertebrates where imprinted genes act early in embryogenesis, influencing important developmental processes. In insects, the few data available on imprinting indicate that it is related to sex determination, as observed in the coccid *Planococcus citri* and in the wasp *Nasonia vitripennis*.

In *Z. pullula* we observed the occurrence of sex specific differences in the methylation pattern and, interestingly, all RAPD PCR profiles showed that feminized male gonads (ovaries) possess the same imprinting pattern of females, despite the male genotype (Fig. 1). This suggests that Wolbachia is not only able to induce a feminization of genetic males, but may also induce the inheritance of female imprinting in gonads of feminized males. This is particularly intriguing since in gonads the parental imprinting is generally erased and re-established on the basis of the parent sex, clearly indicating that feminized males act as true females establishing a female genomic imprinting in their genome.

Given our results we can speculate that Wolbachia’s genome may encode for some proteins that can modulate the expression of the leafhopper’s DNA methyltransferases (Dnmts), in view of the absence of Dnmts in the sequenced genomes of Wolbachia strains isolated from the fly *D. melanogaster* and the nematode *Brugia malayi*, respectively. Nevertheless, it should be noted that there are evidences demonstrating a crucial role of the Wolbachia genotype in determining the type of reproductive alteration, suggesting that different Wolbachia strains may harbor different genes that could explain, for instance, why the Wolbachia strain (wScal) naturally infecting the adzuki bean borer moth *Ostrinia scapulalis* induces male killing, while another strain (wKue) infecting the Mediterranean flour moth *Ephestia kuehniella* induces cytoplasmic incompatibility (CI) in the resident host. At the same time, the same Authors showed that *O. scapulalis* transinfected with wKue, brought to CI, but not male killing indicating that the result of the infection is related to the Wolbachia strain and not to the host. In view of these data, it could be very interesting to wholly sequence the genome of the Wolbachia strain identified in *Z. pullula* to verify, for example, if it encodes for proteins similar to the eukaryotic DNA methyltransferase or, alternatively, if some Wolbachia encoded proteins could interact with Dnmts with a specific interest for Dnmt3, that is generally involved in the establishment of the sex-specific imprinting of the genome.

According to our data, Wolbachia is therefore implicated in reprogramming the host’s genomic imprinting both in somatic tissues and gonads making this maternally transmitted endosymbiont able to induce trans-generational epigenetic changes in the host’s genome.

**References**

1. Stouthamer R, Breeuwer JA, Hurst GGD. Wolbachia *pipientis*: microbial manipulator of arthropod reproduction. Annu Rev Microbiol 1999; 53:71-102.
2. Dobson SL, Bourzis K, Braig HR, Jones BF, Zhou W, Rouset F, et al. Wolbachia infections are distributed throughout insect somatic and germ line tissue. Insect Biochem Mol Biol 1999; 29:153-60.
3. Serbus R, Casper-Lindley C, Landmann F, Sullivan W. The genetics and cell biology of Wolbachia-host interactions. Annu Rev Genet 2008; 42:1-25.
4. Negri I, Pellecchia M, Mazzoglio PJ, Patretta A, Alma A. Feminizing Wolbachia in *Zyginaida pullula* (Insecta, Hemiptera), a leafhopper with an XX/XY sex-determination system. Proc R Soc Lond B 2006; 273:2409-16.
5. Negri I, Franchini A, Gonella E, Daffonchio D, Mazzoglio PJ, Mandrioli M, et al. Unravelling the Wolbachia evolutionary role: the reprogramming of the host genomic imprinting. Proc R Soc Lond B 2009; 276:2485-91.
6. Sunani MA. Imprinting and the initiation of gene silencing in the germ line. Cell 1998; 93:309-12.
7. Bender J. DNA methylation and epigenetics. Annu Rev Plant Biol 2004; 55:41-68.
8. Bongiorni S, Cintio O, Prantera G. The relationship between DNA methylation and chromosome imprinting in the Coccid *Planococcus citri*. Genetics 1999; 151:1471-8.
9. Field LM, Lyko F, Mandrioli M, Prantera G. DNA methylation in insects. Insect Mol Biol 2004; 13:109-15.
10. Beukeboom LW, Kamping A, van de Zande L. Sex determination in the haplodiploid wasp *Nasonia vitripennis* (Hymenoptera: Chalcidoidea): a critical consideration of models and evidence. Sem Cell Dev Biol 2007; 18:371-8.
11. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N, et al. The Wolbachia genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. PLoS Biol 2005; 3:121.
12. Wu M, Sun LV, Vamathavan J, Riegler M, Deboy R, Brownlie JC, et al. Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. PLoS Biol 2004; 2:327-41.
13. Sakamoto H, Ishikawa Y, Sasaki T, Kikuyama S, Tatsuki S, Hoshizaki S. Transinfection reveals the crucial importance of Wolbachia genotypes in determining the type of reproductive alteration in the host. Genet Res 2005; 85:205-10.
14. Karo Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, et al. Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet 2007; 16:2272-80.