

Gene transfers from bacteria and viruses may be shaping complex organisms

Viviane Callier, *Science Writer*

When evolutionary genomicist Richard Cordaux and his team decided to look at the genomes of a puzzling group of pillbugs a few years ago, they set out to test a 30-year-old hypothesis. In 1984, French scientists had shown that sex-determination mechanisms in a particular lineage of *Armadillidium vulgare* were skewed, resulting in 60 to 70% of all births being female (1, 2). Yet, unlike most pillbugs, this population was completely free of the kinds of endosymbiont bacteria known to warp sex ratios in their insect hosts. So why was the skewing effect still present?

The French group had speculated that bacterial DNA in the form of a plasmid might have penetrated the insects' cells and that could explain the lingering bacterial influence. But the group lacked the genomic tools to test their theory. In 2016, Cordaux, at the University of Poitiers in France, and his team reported

a stunning solution to the mystery: One large insertion of DNA from the endosymbiont bacteria *Wolbachia* had integrated itself directly into the pillbug chromosome, essentially creating an entirely new sex chromosome (3). Such a bacteria-to-animal horizontal genome transfer illustrates, says Cordaux, the significant influence of endosymbionts as "agents of evolutionary change in sex-determination systems in animals."

Bacteria are well known to be masters of horizontal gene transfer among their own kind, quickly and seamlessly sharing traits such as antibiotic resistance. But recent work examining animal genome sequences has revealed that gene transfers from bacteria to multicellular organisms are not as rare as previously thought. Moreover, against slim odds, some of these bacterial genes have introduced novel and critical functions to their new hosts. These transfers, say



Researchers found that a large insertion of DNA from the endosymbiont bacteria *Wolbachia* had integrated itself directly into the pillbug chromosome, essentially creating an entirely new sex chromosome via horizontal gene transfer. Image credit: Richard Cordaux.



Wolbachia DNA makes its way into the genomes of pillbugs, fruit flies, and other species with more frequency than previously thought. Image credit: Wikimedia Commons/Scott O'Neill, licensed under CC BY 2.5.

researchers, are an overlooked source of fodder for evolutionary transformation in complex organisms.

Horizontal gene transfer is “definitely turning out to be an important contributor to evolutionary genetics and the metabolic potential of organisms,” says Chris Hittinger, a geneticist at University of Wisconsin–Madison. Hittinger attributes this revelation to two factors: First, in the past few years, the cost of genome sequencing has dropped dramatically. And second, researchers have gotten better at computationally analyzing potential cases to figure out which transfer events are supported by the data and which might be artifacts.

Now, researchers are working to understand just how common these transfers might be as they examine how bacterial genes manage to integrate into the cellular environment of a different and much more complex new host—sometimes even displacing native genes and functions.

A Game of Chance

As sequencing costs dropped in the 2000s, and the number of organisms sequenced rose, researchers started seeing genome sequences that appeared to contain bacterial DNA. They usually chalked this up to bacterial contamination of the sample. But in 2007, genome scientist Julie Dunning Hotopp and her colleagues at the University of Maryland School of Medicine in Baltimore published some of the first evidence that these bacterial sequences really were, at least in some cases, lurking in the genomes of more complex organisms (4).

In that early article, Dunning Hotopp showed that *Wolbachia* DNA had made its way into the *Drosophila* genome. And since then, she’s shown that the fruit flies have quite a lot of *Wolbachia* DNA—about 5 megabases’ worth. That’s about 2% of the *Drosophila* genome and more than three times the DNA in the 1.4-megabase *Wolbachia* genome (5).

How these transfers happen is still not well understood. One possibility is that the process starts with a stressful event that creates a break in the DNA in an organism’s cell. If there’s no intact DNA to serve as a template for repairing the broken strand, the cell searches for whatever DNA is available—perhaps using nearby DNA from bacterial endosymbionts as a sort of Band-Aid on the break, explains Howard

Hughes Medical Institute investigator Harmit Malik, a geneticist at the Fred Hutchinson Cancer Research Center in Seattle, WA. “This is very much one of those chance events,” says Malik, who studies the genetics of evolutionary conflict.

Some recent examples suggest lucky organisms acquired genes that coded for relatively simple metabolic pathways, allowing them to exploit a new food source. For instance, an insect called the coffee berry borer acquired a gene, most likely from gut bacteria, that encodes the enzyme mannanase, which allows the bug to digest a polysaccharide in the coffee berry (6). And parasitic nematodes have acquired cellulases multiple times from a variety of sources that allow them to degrade plant cell walls and parasitize their host plant (7).

Genes for making toxins seem to be another common class of horizontal transfers. New research in bioRxiv from Noah Whiteman’s lab at University of California, Berkeley shows that a bacteriophage-derived toxin gene was horizontally transferred to fruit flies and aphids (8). The researchers hypothesize that the insects use the toxin to defend themselves against parasitoid wasps. Although their own cells are susceptible to the toxin, they manage to deploy it so that only the parasites are harmed (how they accomplish this remains a mystery). A parasitoid wasp, *Nasonia vitripennis*, also appears to have acquired several toxins via horizontal gene transfer and uses the venoms to suppress its host’s immunity and metabolism (9). Thus, horizontal gene transfer might be a significant source of new weapons in an escalating arms race between host and parasite.

But some of the most valuable and widely used agents in the bacterial arsenal actually kill other bacteria.

“How does something that has been out of a lineage for hundreds of millions of years potentially get incorporated into a foreign lineage that it has no experience with? It’s an absolutely fascinating question.”

—Noah Whiteman

A 2014 study of *Wolbachia* revealed an ancient, repeated horizontal transfer of an antibacterial gene that has been incorporated by multiple organisms (10). The gene encodes a lysozyme, an enzyme that a *Wolbachia* bacteriophage uses to break open the bacterial cells.

“What threw us completely into shock, and started an entirely new project, is that this lysozyme had homologs throughout the entire Tree of Life,” says Seth Bordenstein, a microbiologist at Vanderbilt in Nashville, TN, who led the study. It wasn’t just in the bacteriophage and *Wolbachia*. It was also in plants such as ferns and in insect genomes, as well as in archaea that live at the bottom of the ocean near hydrothermal vents.

In 2015, Malik and his colleagues also found that a set of genes encoding powerful antibacterials known as type VI secretion amidase effector (Tae) proteins has been transferred to eukaryotes at least six times (11). The bacteria-killing enzymes are effective against the parasite that causes Lyme disease, and the acquired

genes allow the deer ticks that carry that parasite to make the enzyme.

The life-altering potential for a complex organism in acquiring this kind of bacterial weapon is easy to see, says Malik. “You have a horizontal gene transfer event, and suddenly you have this ready-made antibiotic mechanism to police the bacteria in your gut.”

Against All Odds

Despite the great advantages that bacterial genes may confer on more complex organisms, the odds are still heavily stacked against a transfer producing a functional gene. Researchers are trying to understand how this enormous leap occurs.

Before a bacterial gene can even operate in a eukaryotic cell, it has some major biological hurdles to overcome. It must acquire introns, a promoter sequence, and a termination sequence. It also has to be able to interact fruitfully with existing host genes. “How does something that has been out of a lineage for hundreds of millions of years potentially get incorporated into a foreign lineage that it has no experience with? It’s an absolutely fascinating question,” says Whiteman.

One successful example is *oskar*, a gene that is both necessary and sufficient for germline development in holometabolous insects. *Oskar* is a chimeric gene: Half of it is of bacterial origin, and the other half is of insect origin (12, 13). Because bacteria don’t have a germline development function, the chimeric gene is doing something novel in the insects. It’s also doing something critically important: Without proper germline development, an organism is an evolutionary dead end.

So how can horizontally transferred genes acquire central, critically important roles in their new hosts, even replacing essential host functions such as embryo sex determination, as in the case of the French pillbugs?

Malik suspects the answer may lie in the strong evolutionary pressures that endosymbionts face to

alter their host’s physiology and development to their own advantage. *Wolbachia*, for instance, lives inside the germline and reproductive tissues of approximately 50% of the world’s arthropod species. The bacteria are passed to offspring through their host’s eggs and have evolved multiple strategies to increase the chances of being passed on.

“It’s actually very clear that endosymbiotic bacteria manipulate the germline of insects,” Malik says. “They do so because they want to propagate themselves. They are living and getting transmitted through the oocyte. So there are many bacteria that have manipulated things like the sex determining mechanisms, or that have feminized a genetic male into a female, as a means to increase their transmission.”

Hence, it may not have been difficult for a *Wolbachia* gene to make the functional leap into the genome of French pillbugs. “*Wolbachia* was already interacting with the pillbug’s cells,” explains Cordaux. “Perhaps the integration was just another way to locate the ‘feminization’ gene.” This may also shed light on how *oskar* acquired a novel and essential role in germline development in holometabolous insects some 450 million years ago. The bacterial half of *oskar* might have come from an endosymbiont that was manipulating its host’s germline development in its favor.

All of this helps explain how genes new to a lineage can play key roles in old developmental processes. “We used to have this dogmatic view about genes that are really essential or important—that they are extremely likely to have been conserved since the dawn of time because, of course, they’re essential so we would have not really had an opportunity to mess around with them,” says Malik. “More and more work . . . has really sort of impressed upon folks the idea that, in fact, new genes can be essential. In fact, young genes can acquire essential functions.”

- 1 J.-J. Legrand, P. Juchault, G. Heily, C. Le Bote, Nouvelles données sur le déterminisme génétique et épigénétique de la monogénie chez le crustacé isopode terrestre *Armadillidium vulgare* Latr. *Genet. Sel. Evol.* **16**, 57–84 (1984).
- 2 R. Cordaux, C. Gilbert, Evolutionary significance of *Wolbachia*-to-animal horizontal gene transfer: Female sex determination and the *f* element in the isopod *Armadillidium vulgare*. *Genes (Basel)* **8**, 186 (2017).
- 3 S. Leclercq et al., Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 15036–15041 (2016).
- 4 J. C. Dunning Hotopp et al., Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* **317**, 1753–1756 (2007).
- 5 L. Klasson et al., Extensive duplication of the *Wolbachia* DNA in chromosome four of *Drosophila ananassae*. *BMC Genomics* **15**, 1097 (2014).
- 6 R. Acuña et al., Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4197–4202 (2012).
- 7 W. E. Mayer, L. N. Schuster, G. Bartelmes, C. Dieterich, R. J. Sommer, Horizontal gene transfer of microbial cellulases into nematode genomes is associated with functional assimilation and gene turnover. *BMC Evol. Biol.* **11**, 13 (2011).
- 8 K. I. Verster et al., Horizontal transfer of bacterial cytolethal distending toxin B genes to insects. *Mol. Biol. Evol.*, <https://doi.org/10.1093/molbev/msz146>.
- 9 E. O. Martinson, V. G. Martinson, R. Edwards, Mrinalini, J. H. Werren, Laterally transferred gene recruited as a venom in parasitoid wasps. *Mol. Biol. Evol.* **33**, 1042–1052 (2016).
- 10 J. A. Metcalf, L. J. Funkhouser-Jones, K. Briley, A.-L. Reysenbach, S. R. Bordenstein, Antibacterial gene transfer across the tree of life. *eLife* **3**, e04266 (2014).
- 11 S. Chou et al., Transferred interbacterial antagonism genes augment eukaryotic innate immune function. *Nature* **518**, 98–101 (2015).
- 12 J. A. Lynch et al., The phylogenetic origin of *oskar* coincided with the origin of maternally provisioned germ plasm and pole cells at the base of the Holometabola. *PLoS Genet.* **7**, e1002029 (2011).
- 13 L. Blondel, T. E. M. Jones, C. G. Extavour, Bacterial contribution to genesis of the novel germ line determinant *oskar*. [bioRxiv:10.1101/453514](https://doi.org/10.1101/453514) (25 October 2018).