Genome Evolution: A Bacterium with a Napoleon Complex

New work on an important agricultural pest reveals an unexpected toxin-producing defensive bacterial symbiont. Surprisingly, the symbiont's genome is highly reduced, with genes devoted to polyketide synthesis making up a large fraction of its coding capacity.

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Most animals have beneficial relationships with bacteria. These symbiotic interactions serve many important functions for their hosts: they affect animal development, behavior, reproduction, nutrition, and play various defensive roles [1]. Over the past decade, the interactions between insects and their bacterial symbionts have been relatively well-studied using genomic methods [2,3]. This work has helped clarify the role of the bacteria in these symbioses, and has established parallel patterns of genome evolution in different types of symbionts. For example, insect nutritional symbionts often have highly reduced and stable genomes which encode few genes outside of those needed for the core processes of genome maintenance, transcription, translation, and for nutrient production for the host. Symbionts that affect host reproduction or that play defensive roles also show genome reduction relative to free-living bacteria, but have larger and more dynamic genomes than those from nutritional symbionts. A new study reported in this issue of *Current Biology* from Nakabachi *et al.* [4] describes an insect defensive symbiont that defies easy categorization; its genome shows all the hallmarks of a long-term nutritional symbiont, with the notable exception of the ability to produce many nutrients.

Nakabachi and colleagues start by describing the complete genomes for two endosymbionts in the Asian citrus psyllid, *Diaphorina citri* (Figure 1). Like all other psyllids, *D. citri* has a vertically transmitted and stably associated nutritional endosymbiont called *Carsonella* [5,6]. Previous work has shown that *Carsonella* provides essential amino acids to its host insect, and has a massively reduced genome, ranging in size from 158–166 kilobase pairs (kb) depending on the psyllid species examined [7,8]. At 174 kb, the *Carsonella* genome in *D. citri* is still very small but larger than other previously reported *Carsonella* genomes; the genes encoded in these extra genomic bits are mostly related to essential amino acid biosynthesis. Some lineages of psyllids have been shown to harbor a second endosymbiont, which encodes nutritional genes missing in *Carsonella* in a fascinating complementary fashion [8]. Nakabachi and colleagues also report the presence of a second endosymbiont in *D. citri*, but in this case its genome encodes few genes involved in nutrient production (none are involved in essential amino acid biosynthesis) [4].

This second symbiont, named *Profftella armatura*, has a highly reduced genome of only 465 kb. Remarkably, 15% of the *Profftella* genome is devoted to genes involved in polyketide biosynthesis. Polyketides are a complex family of molecules built from simple acetate and propionate building blocks [9]. These diverse molecules have correspondingly diverse functions. In their natural context, they function as pigments, virulence factors, and defensive compounds, among other roles; in medicine, they have myriad important uses, including as antibiotics and anti-cancer drugs [9]. The genes present in *Profftella* showed similarity to polyketide synthase (PKS) pathways from the defensive bacterial symbionts from sponges and (especially) beetles, leading Nakabachi and colleagues to hypothesize that *Profftella* synthesized a polyketide similar in structure to pederin, the defensive compound made by a symbiont of *Paederus* and *Paederidus* beetles [10,11].

Most genomic work — including, quite honestly, that from my own lab — usually stops here, at the comparative stage. But this new paper goes well beyond sequence comparisons and nails down the structure of the predicted polyketide, which the authors name diaphorin, using mass spectrometry (MS), electron-induced dissociation MS/MS, and nuclear magnetic resonance (NMR). They show that diaphorin is toxic to rat and human cell lines, and that it is present in physiologically relevant concentrations in the insect. While the function of diaphorin is currently unclear, its similarity to pederin suggests that it may be involved in protecting *D. citri* from predators. Overall, the work in this new *Current Biology* paper is a beautiful combination of genomics, biochemistry, and cell biology.

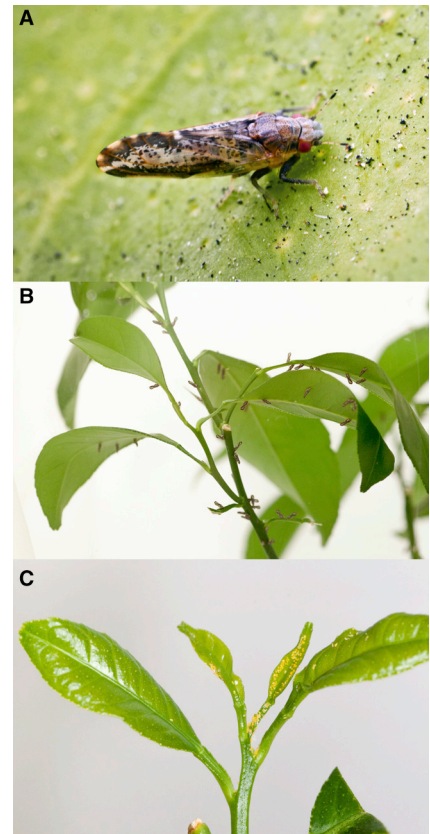Bacteria with small genomes are derived from bacteria with large genomes [12]. Is the large-genome

Figure 1. *Diaphorina citri.*
The Asian citrus psyllid, *Diaphorina citri* (A,B), is an efficient vector of bacteria in the genus *Liberibacter*, which cause huanglongbing, or citrus greening disease (C). Huanglongbing is an incurable and fatal plant disease, and is one of the most serious threats to the global citrus industry. (Photo credits: Mike Lewis, Center for Invasive Species Research, University of California, Riverside.)

ancestor of *Profftella* apparent? Not presently. It is clear that the core of the *Profftella* genome is betaproteobacterial in origin, and that the PKS gene cluster has had a complex history of lateral gene transfer (perhaps tied somehow to the evolution of the PKS gene cluster in *Paederus* beetles) [4]. It seems likely that the PKS genes were transferred to *Profftella* when it was a larger, more dynamic genome, and that selection has maintained these genes over time. That these genes have held fast in the face of such massive genome reduction speaks to their important role in the biology of this system.

While *Profftella* is the first highly reduced protective symbiont genome, other symbiotic bacteria whose genomes encode protective toxins have been sequenced, and comparisons with *Profftella* are instructive. The aphid endosymbiont *Hamiltonella defensa* is typical of many defensive symbionts sequenced to date. Its genome is 2.1 Mb, encodes 2,100 protein-coding genes, has a relatively large number of pseudogenes, and is littered with mobile DNA, insertion sequences, and phage remnants [13]. *H. defensa* is primarily maternally transmitted through eggs, but can also be laterally transferred and is correspondingly found sporadically in distantly related insects [14]. *H. defensa* confers a strong protective phenotype in aphids when parasitoid wasps are present, presumably due to its ability to produce eukaryotic toxins (in an amazing twist, the genes for the biosynthesis of these toxins are actually encoded on an integrated bacteriophage that infects *H. defensa*) [13]. However, it is not found at high frequency in all aphid populations, which is thought to be due to a cost of maintaining the symbiont in the absence of parasitoid pressure [15]. Thus, both the demography and genome structure of *H. defensa* are dynamic.

Although not from an insect, the genome sequence of the tunicate symbiont *Endolissoclinum faulkneri* [16] is an interesting intermediate between *H. defensa* and *Profftella*. While the 1.5 Mb *E. faulkneri* genome is over three times larger than that of *Profftella*, it encodes only 780 functional genes. This corresponds to a very low coding density (57%; typical bacterial genomes have ~85% coding densities), a feature which

has been previously observed in both pathogenic and beneficial endosymbionts [17,18]. Similar to *Profftella,* more than 10% of the functional *E. faulkneri* coding sequence is devoted to the production of a potent polyketide known as patellazole [16]. In contrast to *Profftella*, the PKS gene cluster in *E. faulkneri* is not the result of HGT [16]. In contrast to *H. defensa*, *E. faulkneri* appears very closely linked to a limited group of tunicates, and has correspondingly been shown to be co-evolving with its host [16].

Together the *Profftella*, *H. defensa*, and *E. faulkneria* systems start to bring the genomics of defensive symbiosis into focus. They highlight how differences in ecological interactions can exert profound evolutionary effects on bacterial genomes, and how understanding one affects the interpretation of the other. Perhaps the strongest signal here relates to the stability of the interactions. Bacterial symbionts that are beneficial in certain conditions but are not absolutely required for normal host function, like *H. defensa*, are primarily vertically transmitted but can move horizontally between hosts [2,14]. These bacteria tend to show reduced genomes compared to free-living bacteria, but tend not to be as small as bacteria that are absolutely required by the host [2,3]. Symbionts that are required for normal host biology and that are strictly vertically transmitted tend to have the smallest bacterial genomes, in the range of 150–800 Mb [3,19]. This makes the case of *Profftella* interesting — does the tiny genome of *Profftella* predict that polyketide synthesis is now required for normal functioning of the symbiosis? Nakabachi and colleagues provide some evidence that it might be, as a world-wide PCR screening for *Profftella* in *D. citri* did not reveal a single instance of an uninfected insect [4]. However, just having a small genome does not imply that a symbiont is absolutely required and vertically transmitted by the host; some horizontally transmitted bacterial pathogens, such as *Mycoplasma genitalium,* also have highly reduced genomes [20]. Understanding *Profftella*'s role in the symbiosis will necessitate tracking down the function of diaphorin, and will require

a more careful determination of the bacterium's transmission route and efficiency.

References
1. McFall-Ngai, M., Hadfield, M.G., Bosch, T.C.G., Carey, H.V., Domazet-Lošo, T., Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., et al. (2013). Animals in a bacterial world, a new imperative for the life sciences. Proc. Natl. Acad. Sci. USA 110, 3229–3236.
2. Moran, N.A., McCutcheon, J.P., and Nakabachi, A. (2008). Genomics and evolution of heritable bacterial symbionts. Annu. Rev. Genet. 42, 165–190.
3. Toft, C., and Andersson, S.G.E. (2010). Evolutionary microbial genomics: insights into bacterial host adaptation. Nat. Rev. Genet. 11, 465–475.
4. Nakabachi, A., Ueoka, R., Oshima, K., Teta, R., Mangoni, A., Gurgui, M., Oldham, N.J., van Echten-Deckert, G., Okamura, K., Yamamoto, K., et al. (2013). Defensive bacteriome symbiont with a drastically reduced genome. Curr. Biol. 23, 1478–1484.
5. Thao, M.L., Moran, N.A., Abbot, P., Brennan, E.B., Burckhardt, D.H., and Baumann, P. (2000). Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl. Environ. Microbiol. 66, 2898–2905.
6. Spaulding, A.W., and von Dohlen, C.D. (2001). Psyllid endosymbionts exhibit patterns of co-speciation with hosts and destabilizing substitutions in ribosomal RNA. Insect Mol. Biol. 10, 57–67.
7. Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H.E., Moran, N.A., and Hattori, M. (2006). The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. Science 314, 267.
8. Sloan, D.B., and Moran, N.A. (2012). Genome reduction and co-evolution between the primary and secondary bacterial symbionts of psyllids. Mol. Biol. Evol. 29, 3781–3792.
9. Hertweck, C. (2009). The biosynthetic logic of polyketide diversity. Angew. Chem. Int. Ed. Engl. 48, 4688–4716.
10. Kellner, R.L.L. (1999). What is the basis of pederin polymorphism in *Paederus riparius* rove beetles? The endosymbiotic hypothesis. Entomologia Experimentalis et Applicata 93, 41–49.
11. Piel, J., Höfer, I., and Hui, D. (2004). Evidence for a symbiosis island involved in horizontal acquisition of pederin biosynthetic capabilities by the bacterial symbiont of *Paederus fuscipes* beetles. J. Bacteriol. 186, 1280–1286.
12. Woese, C.R., Maniloff, J., and Zablen, L.B. (1980). Phylogenetic analysis of the mycoplasmas. Proc. Natl. Acad. Sci. USA 77, 494–498.
13. Degnan, P.H., Yu, Y., Sisneros, N., Wing, R.A., and Moran, N.A. (2009). *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. Proc. Natl. Acad. Sci. USA 106, 9063–9068.
14. Russell, J.A., Latorre, A., Sabater-Munoz, B., Moya, A., and Moran, N.A. (2003). Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. Mol. Ecol. 12, 1061–1075.
15. Oliver, K.M., Campos, J., Moran, N.A., and Hunter, M.S. (2008). Population dynamics of defensive symbionts in aphids. Proc. R. Soc. B. 275, 293–299.
16. Kwan, J.C., Donia, M.S., Han, A.W., Hirose, E., Haygood, M.G., and Schmidt, E.W. (2012). Genome streamlining and chemical defense in a coral reef symbiosis. Proc. Natl. Acad. Sci. USA 109, 20655–20660.
17. Andersson, S.G., Zomorodipour, A., Andersson, J.O., Sicheritz-Ponten, T.,

Alsmark, U.C., Podowski, R.M., Naslund, A.K., Eriksson, A.S., Winkler, H.H., and Kurland, C.G. (1998). The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature *396*, 133–140.

18. Burke, G.R., and Moran, N.A. (2011). Massive genomic decay in *Serratia symbiotica*, a recently evolved symbiont of aphids. Genome Biol. Evol. *3*, 195–208.

19. McCutcheon, J.P., and Moran, N.A. (2012). Extreme genome reduction in symbiotic bacteria. Nat Rev. Microbiol. *10*, 13–26.

20. Fraser, C.M., Gocayne, J.D., White, O., Adams, M.D., Clayton, R.A., Fleischmann, R.D., Bult, C.J., Kerlavage, A.R., Sutton, G., Kelley, J.M., *et al.* (1995). The minimal gene complement of *Mycoplasma genitalium*. Science *270*, 397–403.

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Centriole Engagement: It's Not Just Cohesin Any More

The belief that cohesin complexes link mother to daughter centrioles has received substantial experimental support. New studies challenge the primacy of cohesin in centriole engagement and provide a more nuanced view into the mechanisms for centriole disengagement in anaphase.

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The centrosome, the primary microtubule organizing center of the cell, consists of a pair of centrioles associated with a cloud of fibrogranular material known as the pericentriolar material (PCM) which nucleates microtubules. In somatic cells the PCM is associated primarily with the older or mother centriole while in zygotes the PCM cloud can surround both centrioles. The amount of PCM and its microtubule nucleating capacity increases markedly as the cell enters mitosis and rapidly dissipates once the cell is in anaphase [1]. Since centrioles localize the PCM, centrosome duplication is determined by the separation and duplication of centrioles [2]. In preparation for mitosis centrioles and DNA duplicate just once at roughly the same time, suggesting common control. Parallels between chromosomes and centrioles are abundant. The duplication of both are coordinately initiated by a rise in Cdk2–cylin E activity [3], there is a block to reduplication [4], and lastly chromosomes and mother–daughter centrioles separate after the metaphase–anaphase transition which 'licenses' both centrioles to duplicate at the following S phase (reviewed in [5]).

Learning how daughter centrioles are held at their mothers from S phase through metaphase (engagement) and how they become spatially/functionally separated (disengagement) is central to understanding how centrosome duplication is controlled. Studies from the Nasmyth [6] and Dammerman [7] laboratories reported in a recent issue of *Current Biology* provide new insights into centriole engagement and a more nuanced view into their disengagement at the end of mitosis.

Before we discuss these papers, a little information on our present understanding of centriole engagement/disengagement is in order. Using engaged mammalian centriole pairs in *Xenopus* egg extracts, Tsou and Stearns [8] were the first to show that centriole disengagement depends on separase activity. This suggested that centrioles, like chromosomes, are held together by a separase target, logically cohesin complexes at that time. Since then, several studies provided localization and functional observations which established the notion that cohesin complexes engage mother–daughter centrioles until cohesin rings are opened by separase late in mitosis (reviewed in [9]). The singular importance of cohesin in centriole engagement was later solidified by the demonstration that separase-independent opening of cohesin rings at engineered ectopic sites on either of two cohesin subunits was sufficient to cause mammalian centriole disengagement in *Xenopus* egg extracts [10]. The finding that centriole disengagement can occur, albeit substantially late, in separase-null cells did not fundamentally challenge the importance of cohesin complexes in holding centrioles together, because disengagement was dependent upon Plk1 activity early in mitosis [11]. Normally, Plk1 activity disengages chromosome arms early

in mitosis through opening of cohesin rings (the prophase pathway) [12].

However, cohesin is not the only player in centriole engagement. Kendrin/pericentrin B, scaffolding elements of the pericentriolar material, are separase targets and their cleavage is important for centriole disengagement in somatic cells [13,14]. Expression of non-cleavable mutants of kendrin/pericentrin B suppressed centriole disengagement *in vivo* even though cohesin should have been cleaved. Thus, cohesin ring opening may not be sufficient to allow centriole disengagement in somatic cells when the structural integrity of the kendrin/pericentrin component of the PCM remains intact.

Against this somewhat confused background the first study by Oliveira and Nasmyth [6] re-examined the importance of cohesin ring opening for centriole disengagement. The authors used syncytial stage *Drosophila* embryos which were stably arrested in metaphase with engaged chromosomes and centrioles. These embryos expressed cohesin complexes with the Rad21 subunit containing a cleavage site for Tobacco Etch Virus (TEV) protease. This allowed the specific opening of cohesin rings without influencing the integrity of kendrin/pericentrin. First these embryos were co-injected with TEV protease to open the cohesin rings and p27 to inactivate Cdk1 and drive the embryos out of mitosis. Under these conditions both the chromosomes and centrioles disengaged/separated. Then the authors injected only TEV protease to open the cohesin rings while leaving the cells in metaphase. They observed that chromosomes rapidly disengaged and moved apart but the centrioles did not separate. Lastly, injection of p27 alone to drive exit from mitosis resulted in centriole disengagement/separation. Exit from mitosis under these conditions does not lead to prompt