unable to make phenazine. *P. edwinii*, although insensitive to PCA, was engulfed by phenazine-producing bacteria, suggesting that the bacterial–fungal partnership was mutually beneficial. Interestingly, aggregates of *P. edwinii* formed within a specific zone in the *Aspergillus* sp. colonies when exposed to PCA or phenazine-producing species (Figure 1B); such aggregates were not formed when co-cultures were exposed to *Pseudomonas* mutants incapable of producing phenazines. These data supported the hypothesis that phenazines produced by soil bacteria have a strong inhibitory effect on soil fungi, but that this inhibition was alleviated by interactions with bacterial 'friends'.

Rather than stopping at this research juncture, Dahlstrom and Newman then addressed the question of *how P. edwinii* protects *Aspergillus* sp. from phenazines. The authors show *P. edwinii* sequesters PCA in bacterial aggregates, acting as a toxin 'sponge'. This sponge mechanism was tested further by developing genetic methods for *P. edwinii* and showing that mutations in the stress-inducible transcriptional repressor *hrcA* enhanced *P. edwinii* aggregation and sequestration of PCA within the fungal colony. The authors then addressed *why* the accumulation of such a toxic metabolite in the *P. edwinii* aggregates was not detrimental to the fungus. Interestingly, both the *P. edwinii* aggregates and *Aspergillus* sp. colonies showed a drop in redox potential upon exposure to PCA, with fungal colonies acidifying the media, thus triggering PCA accumulation and detoxification in *P. edwinii* aggregates. This cooperative partnership between a bacterium and a fungus amplified the sequestration and detoxification of PCA, to the benefit of both organisms (Figure 1C). Importantly, protection from PCA by P. *edwinii* was not specific to *Aspergillus* sp. but also provided protection to distantly related soil fungi. This study by Dahlstrom and Newman provides a superb road map for others to identify and probe mechanistic hypotheses on functional aspects of bacterial–fungal partnerships in a variety of environments. Importantly, in addition to these interactions shaping microbial communities in the soil, bacterial–fungal partnerships may be particularly important in the rhizosphere, where microbial community composition plays an essential role in plant health and productivity.

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Genome evolution: Minicircular mtDNA and unusual heteroplasmy in a parasitic plant

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Minicircular organelle genomes exist in diverse species but have never been observed in plants — that is, until now. The mitochondrial genome of the holoparasite *Rhopalocnemis phalloides* comprises 21 minicircles, which are extremely heteroplasmic, providing an exceptional example of convergent organelle evolution across disparate lineages.

"Everything comes in circles. […] The old wheel turns, and the same spoke comes up. It's all been done before, and will be again." (Arthur Conan Doyle — *The Valley of Fear*).

The mitochondrial genomes of parasites are renowned for having unconventional architectures, ones that can make your head spin[1]. The analysis of the mitochondrial genome of the holoparasitic plant *Rhopalocnemis*

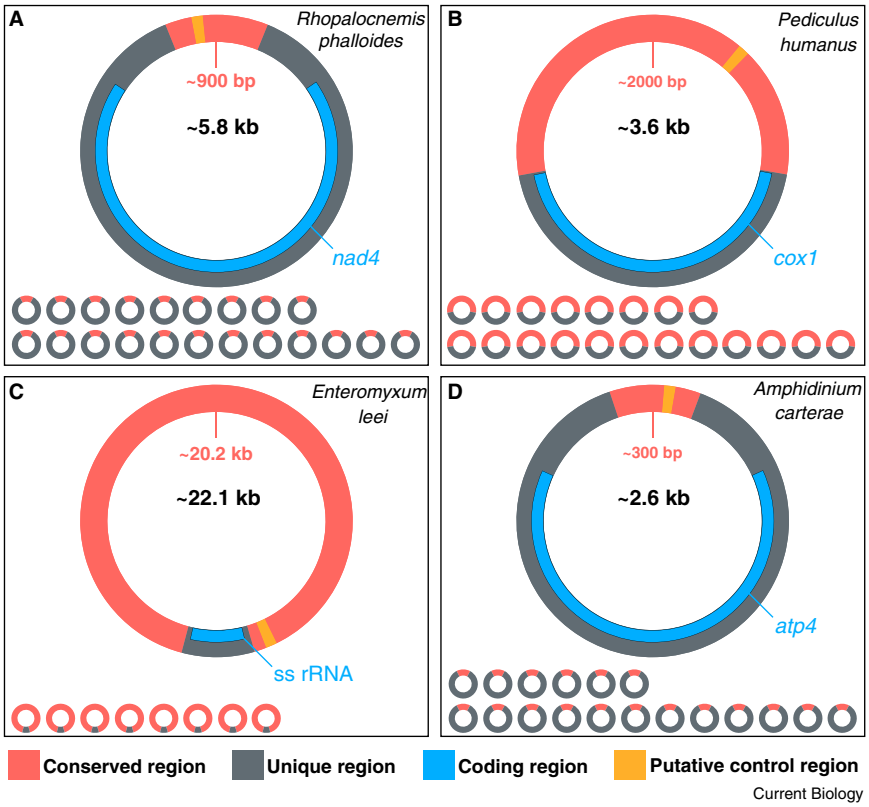

Figure 1. Basic architecture of minicircular organelle chromosomes from selected species.
(A–C) Schematics of minicircular mitochondrial DNA from the indicated species. (D) Schematic of minicircular plastid DNA from *Amphidinium carterae*. The large chromosomes depicted in the centre of the panels represent simplified genome maps of randomly selected minicircles and include overall chromosome sizes and sizes of conserved regions, as well as indicating examples of genes encoded on these minicircles. The small chromosomes at the bottom of the panels represent the total number of minicircles within each indicated species, including empty minicircles and, for *A. carterae*, microcircles (minicircles that are <500 bp in size). Note that variable regions and semi-conserved regions are not shown.

phalloides — reported in this issue of *Current Biology* by Yu *et al.*[2] — will literally have you seeing circles. The mitochondrial genome of this non-photosynthetic species is arranged into 21 miniature circular chromosomes of ~4–8 kb with between 0 and 4 genes apiece and is unlike any other plant mitochondrial DNA (mtDNA) observed to date. But it is not the uniqueness of this genome that makes it so interesting, but rather its similarities to minicircular organelle DNAs of other parasites (and non-parasites) from disparate lineages. You would be forgiven if when reading Yu *et al.*[2] you experience a sense of *déjà vu*. The mtDNA architecture of human body lice (*Pediculus humanus*), for example, is nearly identical to that of *R. phalloides*: 20 minicircular chromosomes of 3–4 kb each carrying ~1–3 genes[3] (Figure 1A,B).

If that doesn't leave you scratching your head, how about this: the mitochondrial genome of the myxozoan parasite *Enteromyxum leei* (Cnidaria) is also fragmented into small circular chromosomes[4] (Figure 1C), as are those of the parasitic mesozoan *Dicymea misakiense*[5], the parasitic nematode *Globodera pallida*[6], and the euglenozoan *Diplonema papillatum*[7]. And don't forget the textbook example of maxi- and mini-circular mtDNA from the parasitic kinetoplastid *Trypanosoma brucei*[8]. Chloroplast genomes, too, can exist in minicircular forms, specifically those of peridinin-containing dinoflagellates (such *as Amphidinium carterae*), which contain about a dozen 2–5 kb plasmid-like chromosomes[9] (Figure 1D).

Perhaps what is most intriguing about the convergent evolution of minicircular genomes across diverse lineages is the organization of their coding and non-coding elements (Figure 1A–D). A recurring theme is that they contain a conserved region, which is typically non-protein-coding and repeated on all the minicircles within a species, as well as a unique region, which usually contains 1–5 genes, or a fragment of a gene, or in some cases no gene at all[1,3,4,7–9] — that is, an 'empty' minicircle.

In the *R. phalloides* mtDNA[2], the ~900 bp conserved region is identical among the 21 chromosomes and contains a long palindromic repeat, which can be folded into a stem-loop structure and is believed to be the origin of replication, paralleling observations of other organelle minicircles[3,4,8,9]. Bookending the conserved region are two blocks of sequences (~200–450 bp), each containing hypervariable and semi-conserved regions. These blocks lead directly to the unique region (~3.5–7 kb) of the minicircles, which collectively contain the typical set of mitochondrial genes from an angiosperm. Altogether, the minicircles have a total length of ~130.7 kb (45% GC), meaning *R. phalloides* currently has the second smallest known mitochondrial genome from a vascular plant[2]. And keep in mind that this length includes two empty minicircles (chromosomes 16 and 18), which are annotated as being devoid of genes.

What really makes the *R. phalloides* mitochondrial genome stand out when compared with other well-studied organelle genomes, including other minicircular DNAs, is its extreme heteroplasmy. Close inspection of the *R. phalloides* sequencing reads, which came from a single individual collected in Daweishan National Forest Park in China, revealed large numbers of insertion/deletion mutations or indels (as well as more complex forms of variation), many of which were in the unique region of the chromosomes. All 21 unique regions displayed dozens of different variants, including a whopping 143 indels across protein-coding regions.

To be clear, this heteroplasmic variation is not simply silent 'background noise'. It can have serious functional consequences. Take the gene encoding ATP synthase 4 (*atp4*), for instance. Three

indel variants of this gene were identified in the Illumina sequence data, the existence of which were further verified using PCR and clonal sequencing. Here's the kicker: Yu *et al.*[2] state that "the majority of the amplified products (79 of 100 colonies) contain[ed] frameshifts and premature stop codons, indicating that functional *atp4* copies [were] the minority". Before you ask, yes, the indels were also present in the mitochondrial transcriptome. In fact, all the variants found in the DNA sequencing reads were also detected in the RNA sequencing data, indicating that this parasitic plant is generating large quantities of putatively non-functional transcripts.

It is not obvious what is responsible for this extreme error-prone heteroplasmy. But two nuclear-encoded, mitochondrial-targeted enzymes involved in DNA replication, repair, and recombination (*WHY2* and *OSB2/3/4*) that are typically found in angiosperms[10,11] are missing from the *R. phalloides* transcriptome. Indeed, it is becoming increasingly apparent that the origins of bizarre and unconventional organelle genomic architectures often have their roots in absent or aberrant components of the DNA maintenance machinery[1,10–12].

The work of Yu *et al.*[2] underscores a trend in the field of organelle genomics: the increasing usefulness of long-read DNA sequencing reads for deciphering complicated and dynamic genomic architectures[13], and not just large genomes. Although the authors were unable to capture individual minicircles within their PacBio DNA sequence data as the insert size was too large, they did identify hundreds of reads comprising concatenated forms of the mitochondrial chromosomes. Most of these reads contained homo-concatemers, that is, the same chromosomal unit in a head-to-tail configuration. Some, however, had more complex organizations, including permutations of two or three different chromosomal units. The existence of these homo- and hetero-concatemers was confirmed by Southern blotting, as was the majority presence of monomeric forms. Together, these data suggest that the minicircles are highly recombinogenic and replicate via a rolling-circle or T4 phage-like mechanism[14]. How plant mtDNAs replicate is a long-standing mystery[15]. Just by chance of it having miniature chromosomes — the concatenated forms of which were easily captured in long reads — the *R. phalloides* mitochondrial genome has provided a key insight into this issue.

When discussing unusual genomes in parasitic plants and algae, the first thing that normally comes up is plastid DNA. So, what of the *R. phalloides* plastid genome? Like the plastid genomes of many other holoparasites, it is severely reduced, having lost all genes related to photosynthesis, and is exceptionally AT-rich (87%)[16]. But, unlike its mitochondrial counterpart, the plastid DNA is not fragmented into minicircles, nor does it display any detectable heteroplasmy. Thus, in a competition of "Which genome is weirder?", I would argue that the mitochondrial genome comes out on top.

Parasitism has evolved no fewer than a dozen times in flowering plants, encompassing ~4,750 species across 292 genera[17]. Only a handful of these species have had their mitochondrial genomes characterized in any great detail. If the mitochondrial genome of *R. phalloides* is anything to go by, parasitic plants may have a lot more to teach us about the extremes of organelle genome evolution. On this front, it is noteworthy that the holoparasitic plants *Lophophytum mirabile* and *Ombrophytum subterraneum*, which belong to the same family as *R. phalloides* (Balanophoraceae), have multi-chromosomal mitochondrial genomes[18,19]. However, the circular-mapping mitochondrial chromosomes of these two species are quite large (total length >710 kb) and don't bear the standard hallmarks of minicircles. Nevertheless, they are exceptional in their own right. Keep your eyes on the Balanophoraceae. I'm willing to wager ten minicircles that this family will continue to awe us with its extraordinary organelle genomes for many years to come.

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Axis specification: Breaking symmetry with a myosin patch in the egg

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Drosophila **anterior–posterior axis specification occurs in the oocyte, but the initial symmetry break has been unclear. A new study reveals that a posterior domain of cortical myosin is induced with unique post-translational modification and dynamics and that this domain recruits downstream posterior determinants.**

The major axes of the animal body plan arise from a break of symmetry. Establishing which sides of an early embryo are to become head, tail, back, or belly is needed for the spatial organization of cell differentiation and tissue morphogenesis. In some organisms, the initial symmetry break occurs across early cell populations from the interplay of intercellular signaling and transcriptional change[1]. In other organisms, symmetry is broken at the one-cell stage[2]. Pattern generation within a cell, more commonly described as 'cell polarization', is critical to cellular functions ranging from cell division and cell migration to epithelial cell secretion and neuronal signaling[3]. For all these activities, cells pattern their cortex, a thin network of actin filaments and additional proteins underlying the plasma membrane[4]. A new study by Doerflinger, Zimyanin, and St Johnston[5] published in this issue of *Current Biology* shows that anterior–posterior axis specification in *Drosophila* is also initiated by patterning of the cell cortex (Figure 1).

Drosophila is a key model for understanding the principles of embryonic axis determination[2,6]. Each year, introductory developmental biology courses describe the opposing anterior–posterior gradients of Bicoid and Nanos and explain how this positional information is transformed into the segmented structure of the developing larva. A curious mind then asks how these gradients are established and, in turn, how the upstream cues are positioned. What is the initial symmetry break? In *Drosophila*, the oocyte gains an anterior–posterior axis before fertilization while it develops in conjunction with supportive nurse cells and encased within a surrounding follicular epithelium. Previous studies have shown that the initial symmetry break involves signaling from posterior follicle cells and an accumulation of the kinase Par-1 at the posterior cortex of the oocyte, but the link between the follicle–oocyte signaling and the oocyte Par-1 polarization has been elusive.

Doerflinger *et al.*[5] pursued the missing link by conducting a screen of animals with a mutant germline. A group of mutants lacking the symmetry break all carried mutations that mapped to a single gene encoding a molecular chaperone, Unc-45, which typically aids the expression of myosin family members. Consistent with this role, the mutant oocytes also lacked cortical localization of non-muscle myosin II (hereafter called myosin). To test the role of myosin directly, Par-1 localization was examined in mutants of myosin regulatory light chain. These myosin mutants lacked the normal enrichment of posterior Par-1 and showed mis-localization of factors that Par-1 would otherwise recruit. In normal oocytes, myosin displayed a modest elevation of protein levels at the posterior, but, more strikingly, a di-phosphorylated form of myosin regulatory light chain was highly enriched in a posterior patch. This di-phosphorylation was lost with disruption of the follicle cell signaling but was retained with the removal of Par-1. Thus, a specific myosin network formed downstream of the follicle–oocyte signaling and upstream of the oocyte Par-1 polarization.

To understand the role of the myosin patch, Doerflinger *et al.*[5] pursued the functional relevance of the di-phosphorylation of myosin regulatory light chain. Myosin can be activated by the phosphorylation of its regulatory light chain at one amino acid residue, and this activity is then boosted by additional phosphorylation of a neighboring residue[7]. The relevance of the di-phosphorylation was investigated by mutating the second residue and by inhibiting the responsible kinase, myosin light chain kinase. Either perturbation disrupted the initial oocyte