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Series

A cross-eyed geneticist's view
II. Riddles, wrapped in mysteries, inside … mealybugs

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1. Mealybugs – An introduction

Mealybugs (Insecta: Hemiptera: Pseudococcidae) were described as 'a bug in a bug in a bug' (Szabó *et al*. 2016) because a γ-*Proteobacterium* lives inside a β-*Proteobacterium*, which lives inside an insect cell. Make that 'a bug in a bug in a bug in a bug'. In the citrus mealybug, *Planococcus citri*, the outermost bug (bug 1) is the scale insect in which the females are agricultural pests that suck the host plant's phloem sap. They are 'soft, often elongate or oval, and usually attached to plant surfaces … covered with a mealy or cottony wax secretion' (Thao *et al*. 2002; Ross and Shuker 2009). The innermost, bug 4, is the γ-*Proteobacterium*, *Moranella endobia* PCIT ('PCIT' indicates it is the *P. citri* isolate), which resides inside bug 3, the β-*Proteobacterium*, *Tremblaya princeps* PCIT, which resides in an insect cell called the bacteriocyte, and several bacteriocytes together constitute the bacteriome (also called the mycetome), a structure within the body cavity of bug 1 (von Dohlen *et al*. 2001). The bacteriocytes are genetically different from the rest of the insect cells, and therefore the bacteriome (bug 2) is distinct from the sexually reproducing bug 1 (Normark 2004a). While bug 1 consists of diploid cells descended from the fertilized oocyte (zygote I), the PCIT bacteriocytes are pentaploid and are derived from a 'zygote II', formed by fusion of zygote I-derived diploid cells with triploid cells descended from the fusion of the meiotic polar bodies (see below). An even more outlandish relationship between bugs 1 and 2 was reported recently in the whitefly *Bemisia tabaci*. Here, the bactericytes were seemingly immortal polyploid cells passed down the maternal lineage along with their bacterial passengers, and they were only remotely related to the other cells of the insect body (Luan *et al*. 2018). The mealybug's resemblance to a Russian matryoshka doll (López-Madrigal *et al*. 2013) brings to mind Winston Churchill's clever (though sanctimonious) description of Communist Russia as 'a riddle wrapped in a mystery inside an enigma'.

Here is riddle 1: Why do bacteriocytes have a different genotype than the rest of the cells of the insect's body? In the diploid cells of males, the paternally derived chromosomes are heterochromatic and not passed on into the sperm, whereas the paternal genome is euchromatic and expressed in both male and female bacteriomes. Additionally, sibling mealybugs share 100% of their bacteriocyte genomes but only 75% of the genome of their other cells (see below). Normark (2004b) suggested that the 'gender crypsis' in bacteriocytes makes it difficult for the symbiotic bacteria to infer their host's sex, and thus might preserve male embryos from bacterially-induced elimination. Males are a dead end from the endosymbiont's point of view, and in several mealybug lineages the bacteria have evolved strategies to induce male-killing or parthenogenesis, and to thereby increase their representation in the germline. In parthenogenesis, embryos develop from unfertilized egg cells. Uzi Nur (see Normark and Ross 2010) categorized seven types of parthenogenesis in mealybugs, including 'obligate automictic thelytoky', in which '… the eggs develop without fertilization and give rise only to females [although] diploidy is restored by the fusion of two haploid nuclei during the second cleavage', thus making the individual insect homozygous for all loci (Nur 1971).

Riddle 2: Do bacteriocytes in obligate automictic thelytokous mealybugs have a different genotype than the other cells?

The primary and secondary bacterial endosymbionts of *P. citri*, *T. princeps* and *M. endobia* move during embryonic development from the mother's to the embryo's bacteriome. *T. princeps* has among the smallest bacterial genomes (∼139 kb). Many functions missing from it are complemented by genes in the *M. endobia* and *P. citri* genomes, and the *P. citri* genome also includes genes acquired ancestrally via horizontal transfer from other bacteria (McCutcheon and von Dohlen 2011). This 'patchwork' of endosymbiont genes, host genes, and genes acquired by the host from other bacteria encode enzymes that synthesize nutrients missing from the plant sap. Comparison of *P. citri* with *Trabutina mannipara*, the tamarisk tree mealybug, whose innermost bacterium, *Trabutinella endobia*, represents a novel γ-*Proteobacteria* lineage, revealed that gene functions partitioned in a similar manner between the symbiotic partners in the two systems (Szabó *et al*. 2016). This indicated that the horizontally acquired genes were present in the common ancestor of *P. citri* and *T. mannipara*, and that subsequently different γ-*Proteobacteria* symbionts independently infected *Tremblaya* in the two systems. Endosymbionts are prevented from exchanging genes with other lineages and hence undergo degenerative evolution with time, which traps the host into a symbiotic 'rabbit hole'. The transfer of endosymbiont genes to the host nuclear genome can expose them to the sexual cycle and thus slow down the descent into the rabbit hole, whereas endosymbiosis by novel γ-*Proteobacteria* can afford the host an exit from the rabbit hole, and even allow it to access a previously inaccessible food source. Husnik and McCutcheon (2016) have suggested that given that its genome is so whittled down, and that so many of its envelope proteins are synthesized by the host bacteriocyte, Tremblaya (bug 3) is not quite the β-*Proteobacterium* that its ancestor was, and hence it might not be quite as challenging to replace the innermost bacteria. In other words, the uniqueness of the 'prokaryote within a prokaryote' relationship of bugs 3/4 might be overblown.

Riddle 3: What adaptations enabled such nesting?

2. Meiosis, inverse meiosis, and bacteriocytes

To better appreciate the genetic difference between the bacteriocyte and the rest of the mealybug it is worth briefly reviewing meiosis. Meiosis begins with a diploid cell, containing two copies of each chromosome, one from the father and the other from the mother, and ends with the production of four haploid cells containing only one copy of each chromosome. In females the diploid cell that initiates meiosis is called the primary oocyte, and in males it is the primary spermatocyte. At the onset of meiosis, the chromosomes are replicated, and in the best studied organisms the newly made sister chromatids are held together by the kinetochore complex that forms on the centromeric DNA sequences. The first division of meiosis segregates homologous (i.e. paternal and maternal) sister chromatid pairs to opposite spindle poles (figure 1, sequence a, b, c, d). Since each daughter nucleus receives a haploid number of chromosomes, it is called a reductional division, although the sister chromatids still remain attached at their centromeres. In females the reductional division produces a large secondary oocyte and a small polar body I (PBI), while in males it produces two equal-sized secondary spermatocytes. The next division of meiosis is equational, wherein the sister chromatids lose kinetochore cohesion and segregate to opposite poles. Equational division of the secondary oocyte produces a large ovum and a small polar body II (PBII), and if PBI also undergoes equational division, two additional PBII are produced. Equational division in males produces equal-sized spermatids, which then differentiate into the spermatozoa.

The sequence of meiotic divisions is inverted in mealybugs (figure 1, sequence a, b, c', d'). The first meiotic division is equational and the second, reductional (Chandra 1962; Bongiorni *et al*. 2004). Additionally, Bongiorni *et al*. (2004) showed that the paternal chromosomes are eliminated during male meiosis, because the second meiotic spindle is unipolar and binds only to the euchromatic chromosomes; thus, all sperm transmit only the maternal chromosomes, and hence are genetically identical. The secondary oocyte and PBI are diploid, and the ovum, PBII, and sperm are haploid. Fusion of the sperm and egg produces a diploid zygote (zygote I). In parallel, the diploid PBI and haploid PBII fuse to form a triploid polar nucleus, which then divides several times and the division products fuse with (diploid) cleavage nuclei derived from zygote 1 to produce the pentaploid bacteriocytes. Bacteriocytes of full siblings, regardless of whether they are male or female, share 100% of their genomes. In contrast, the bug 1 cells of full siblings share only 50% of the maternal genome, and although they inherit the same paternal genome, this fraction becomes heterochromatic and is not expressed in males, whereas it is euchromatic and expressed in females. We still do not know what cues make an embryo develop as either a male or a female. Sharat Chandra's seminal demonstration of inverse meiosis in mealybugs employed a genetic trick to generate triploid females by mating their mothers with males irradiated with gamma-rays. Presumably, the damaged paternal chromosomes in zygote I cause its development to be aborted, and the triploid cells derived from fusion of the polar nuclei take on the formation of both the embryo (bug1) and bacteriome (bug 2). Almost all of the progeny from Sharat Chandra's crosses were triploid females and in them bug 2 was probably either a triploid or a tetraploid. It would be interesting to examine whether the bacteriocyte/host cell interaction in the triploid females is altered in some way.

Triploid *P. citri* has 15 chromosomes, and during reductional division a triad can segregate as a monad and a dyad

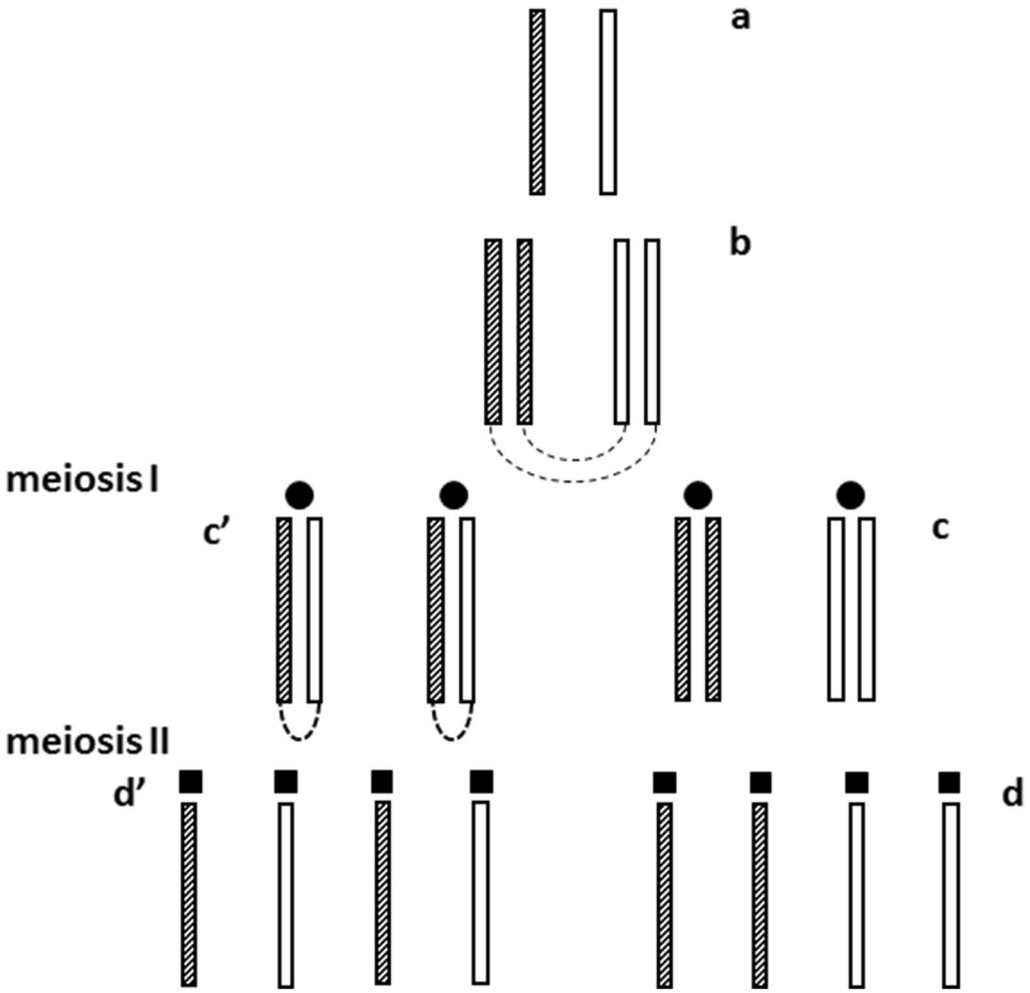

Figure 1. Meiosis (**a**, **b**, **c**, **d**) and inverted meiosis (**a**, **b**, **c**', **d**'). The paternal and maternal chromosomes (**a**, hatched and open bars), also known as homologous chromosomes, are replicated (**b**). In the first division of meiosis (meiosis I; **c**) the homologous chromosomes segregate to opposite poles of the first spindle (filled circles), and in the second division (meiosis II, **d**), the sister chromatids of each homologue separate and go to opposite poles of the second spindle (filled squares). In contrast, in the first division of inverted meiosis (meiosis I; **c**') homologous non-sister chromatids (one hatched + one open bar) segregate to opposite poles of the first spindle, and in the second division (meiosis II, **d**'), the homologous chromosomes segregate to opposite poles of the second spindle. Dashed lines in **b** and **c**' represent chromatin threads that terminally link homologous non-sister chromatids until metaphase II, and they become separated only in meiosis II.

going to opposite spindle poles. The reductional division was identified as the one which showed segregation of 5 and 10, 6 and 9, or 7 and 8 chromosomes to opposite poles, and Sharat Chandra's cytological observations revealed this happened in the second division in meiosis. More recently inverse meiosis was shown to occur in a few plant species (Heckmann *et al*. 2014). Cytologically, the first division (equational) was shown to be different from a mitosis (Marques *et al*. 2016), despite the fact that both apparently achieve the same outcome, viz. production of diploid daughter cells. The common denominator in species showing inverse meiosis is their chromosomes are holocentric (i.e. the centromeres are diffuse), rather than monocentric as in humans and most 'model' systems. This begs the question – why do some species have holocentric chromosomes? And brings me to my mystery – what, if anything, precludes the evolution of a species in which some chromosomes segregate via regular meiosis while others are holocentric and segregate via inverse meiosis?

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