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NEWS AND COMMENTARY

Unexpected diploid males

Check out these males

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Some insect orders are well known for a particular characteristic: for example, coleopteran species (beetles) are numerous; dipterans (flies, mosquitoes) have only one pair of wings; and isopterans (termites) are social. The trait characterizing all members of the orders Thysanoptera (thrips) and Hymenoptera (wasps, bees, ants) is the haplodiploid genetic system: females are diploid (they have two sets of chromosomes and are usually produced from fertilized eggs) whereas males are haploid (they have only one set of chromosomes, and are usually produced from unfertilized eggs). Several models have been proposed to explain sex determination in this system, and these are attributed to specific superfamilies. Models include paternal genome elimination, genomic imprinting and complementary sex determination (CSD) (reviewed in Heimpel and de Boer, 2008). The CSD model involves locus-dependent sex determination: females are heterozygous at these loci, whereas males are hemizygous. Under CSD, fertilized diploid eggs can result in diploid males if they are homozygous at the relevant loci, perhaps due to inbreeding. In this issue, Giorgini *et al.* (2009) present another example of diploid males, in the parasitoid wasp *Encarsia hispida* of the Chalcidoidea family. But this superfamily does not exhibit CSD, and diploid males were thought to be virtually absent. This surprise is just the tip of the iceberg, digging down reveals a much more complicated picture.

Cold

Under a simple scenario without CSD, in which diploidy alone is necessary for female determination, diploidy must be recovered either by fertilization or by another means of restoration. Symbiotic bacteria have been shown to be involved in the latter category. Bacteria-induced thelytokous reproduction—in which virgin females produce solely female offspring—was first discovered in the parasitic wasps of the genus *Trichogramma* in 1990. Stouthamer *et al.* (1990) demonstrated that feeding virgin, parthenogenetic females with antibiotics resulted in the production of male progeny. Since then, a number of other cases of thelytoky have been associated with the α-proteobacterium *Wolbachia*, the super-symbiont of arthropods (Werren *et al.*, 2008). In recent years, another α-proteobacterium, *Rickettsia,* and the Bacteroidetes *Cardinium* have also been associated with thelytoky (Hagimori *et al.*, 2006 and Zchori-Fein *et al.*, 2004, respectively). The diploid males discovered by Giorgini *et al.* (2009) are a result of antibiotic treatment of *E. hispida*, which naturally carry a thelytoky-inducing *Cardinium*. Interestingly, bacteria-induced thelytoky has only been discovered in non-CSD hymenopterans, and the common assumption has been that the symbiont is simply involved in producing females from unfertilized, incipient male eggs by restoring diploidy.

Getting colder

Until now, conventional thinking followed the idea that when thelytoky-inducing symbionts are removed, the diploidy-restoration mechanism is interfered with and unfertilized eggs result in haploid males. In light of this assumption, cytological observations revealed that the mechanism by which diploidy is restored in *Wolbachia*-infected species is gamete duplication (the collapse of the first embryonic mitotic division) (for example, Stouthamer and Kazmer, 1994) and in *Rickettsia*-infected *Neochrysocharis formosa* it is apomixis (reductive meiosis does not occur; Adatchi-Hagimori *et al.*, 2008). It was assumed that the males produced as a result of antibiotic treatments were haploid and so their ploidy status was not actually determined in any of the pertinent studies reported so far.

Freezing

Other models of sex determination in non-CSD haplodiploids employ genomic imprinting, in which the maternal chromosome set is marked in some location in a way that allows the egg to develop into a male. When a non-imprinted chromosomal set (of paternal origin) is present, it compensates for the imprinted location and the embryo develops into a female (Beukeboom *et al.*, 2007). The report by Giorgini *et al.* (2009) suggests that imprinting present in a restored diploid genome may also allow male development (whatever the mechanism of restoration of diploidy) (Figure 1). Indeed, as stated in the title of the Giorgini *et al.* (2009) study, *Cardinium* in *E. hispida* does not restore diploidy but acts as a feminizer of an already diploid product. Feminization has been attributed to *Cardinium* in a haplodiploid mite in a previous study (Weeks *et al.*, 2001). Although a specific form of genomic imprinting has been suggested to play a role in sex determination in the chalcidoid wasp *Nasonia vitripennis* (Beukeboom *et al.*, 2007), it is not known whether this mechanism occurs in most Chalcidoidea or whether antibiotic-induced males are always diploid. If they are, this would fit with the notion that thelytoky-inducing symbionts may only be able to invade species where a diploidy-restoration mechanism already exists, and feminize the diploid embryo by controlling the imprinting mechanism such that it develops into a female rather than a male (Figure 1). The assumption that symbionts act on imprinting mechanisms, and not on diploidy-restoration mechanisms, would explain why they cannot become established in CSD hymenopterans.

Sub-zero

The evolution of haplodiploidy has been studied and discussed extensively and a model of symbiont involvement has been suggested (Normark, 2004 and references therein). Giorgini *et al.* (2009) hypothesized that *Cardinium* may be involved in the reversal from haplodiploidy to the ancestral state of diplodiploidy. However, a problem with this proposal is that diploid males in Hymenoptera with CSD are in most cases inviable or sterile, and sterility is also the norm amongst antibiotic-induced males. Moreover, the function of *E. hispida* diploid males was not checked.

Studying male function and the mechanism of diploidy restoration in *E. hispida*, as well as elucidating ploidy in other antibiotic-induced types of male, can either support the arguments above and the claim that thelytoky-inducing bacteria are feminizers, or perhaps suggest that *Cardinium* is a unique reproductive manipulator bacterium within haplodiploid systems. Until

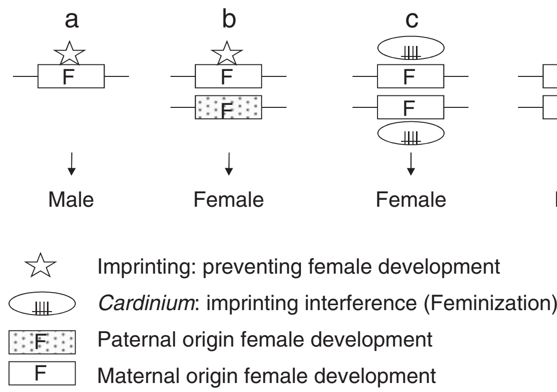

Figure 1 Model for genomic imprinting interference (diploid product feminization). (a) Normal haploid development. Maternal gene for female development is blocked by imprinting. (b) Normal diploid development. The normal paternal gene compensates for the blocked maternal gene. (c) *Cardinium*-infected genetically fixed diploid interferes with imprinting and causes female development. (d) Removal of *Cardinium* results in imprinting of both sets of maternal genes, leading to male progeny.

a definitive sex-determination mechanism in more bacteria-infected non-CSD hymenopterans is established, don't forget to check out these males.

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