

Molecular basis of sex determination in haplodiploids

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Sex in many species of Hymenoptera (ants, bees and wasps) is determined by a single locus that is heterozygous in females and hemizygous in (haploid) males. Beye and colleagues have now cloned the *csd* locus in the honeybee *Apis mellifera* and provide functional evidence that this gene is the primary switch in the sex-determination cascade of honeybees and possibly all Hymenoptera.

It has been known for 150 years that male honeybees develop from unfertilized eggs whereas females develop from fertilized eggs (Figure 1). This seemingly odd mechanism for determining male/female development is actually quite widespread, having independently evolved ~15 times in insects and mites alone (Figure 2; [2]) and occurring in perhaps 20% of animal species [3]. In honeybees *Apis mellifera*, sex is determined by the actions of a single complementary sex-determining (*csd*) locus that is heterozygous ($A_1 A_2$) in females and hemizygous (A_1 or A_2) in haploid males ([4], Figure 1). Diploid individuals that are homozygous at the *csd* ($A_1 A_1$) or ($A_2 A_2$) are males, but are eaten by the workers at the first larval instar [5]. After an extensive search, a new paper by Beye *et al.* [1] reports the cloning and sequencing of the *csd* of the honeybee. Knowledge of the honeybee *csd* should pave the way for comparative studies of the mechanisms of sex determination across haplodiploid species, helping us to better understand shared mechanisms for sex in this diverse and important group.

Finding and characterizing the gene

Beye *et al.* [1] created an inbred honeybee cross in which 50% of the offspring failed to mature because they were diploid males. By fine-scale mapping of a region between two genetic markers that co-segregate with diploid male production, the authors identified a 13-kb region that was always heterozygous in the females of the inbred cross [6]. Using PCR analyses of cDNA isolated from 0–30-hr-old embryos, with primers designed within the 13-kb region, a 1.5-kb transcript was identified as *csd*. Genomic sequencing and Southern blotting confirmed that the locus spans 9.3 kb, comprises nine exons and encodes a 385 amino-acid protein. The most convincing piece of evidence that *csd* is the primary sex-determination signal [1] comes from the repression of *csd* activity in early development by RNA interference. Injecting a double-stranded (ds) *csd* RNA transcript into fertilized eggs transforms genetic females into males. Male larvae injected with *csd* dsRNA were not

affected, showing that *csd* function is not required for normal male development.

The encoded protein (*csd*) contains an arginine–serine-rich domain and a proline-rich region in the carboxy-terminal region, but lacks an RNA-recognition motif (RRM) in the amino-terminal region. This suggests that it is an atypical member of the SR family of proteins, other members of which have been shown to function in RNA binding and mRNA processing [7]. Although *csd* appears to be unique, it shows sequence similarity to the *tra* (*transformer*) protein of *Drosophila*, which is also involved in sex determination [8].

As identical full-length transcripts of *csd* are detected in both sexes soon after cell formation begins (~12 hr of development) and are then found continuously throughout development, Beye *et al.* excluded differential expression and processing of *csd* as the basis of sex determination. Instead, *csd* activity is controlled after protein synthesis, which is consistent with the current model for *csd* [4], which calls for a dimeric protein that functions only when

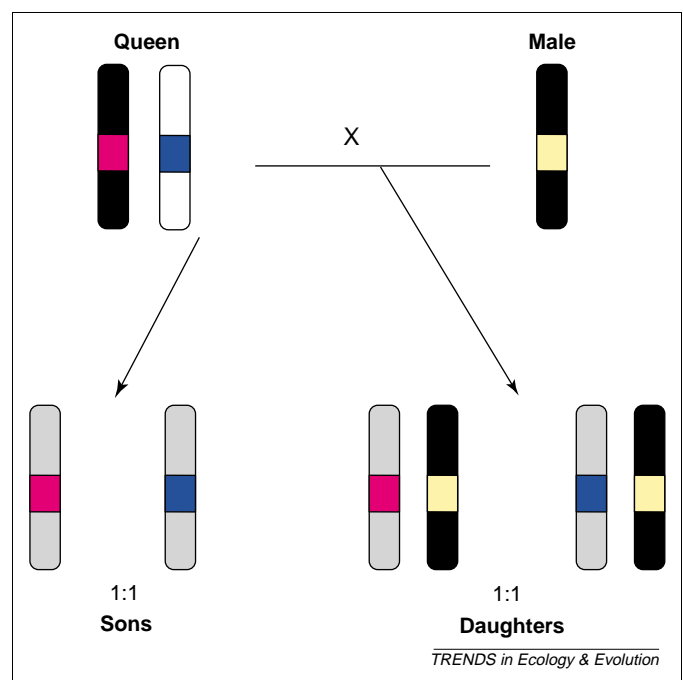


Figure 1. Haplodiploidy and sex determination. The diagram shows a diploid queen and her haploid mate, holding three distinct alleles at *csd* (pink and blue for the queen; yellow for her mate). The queen produces haploid male offspring (showing mendelian segregation during meiosis and a 1:1 ratio of her two *csd* alleles) and diploid female offspring, whose maternal genomes segregate at *csd* and other genes, but whose paternal genomes are identical copies with each other and with their father. Non-viable diploid males occur if there is a match at the *csd* locus (e.g. two 'yellow' alleles). Abbreviation: *csd*, complimentary sex determiner.

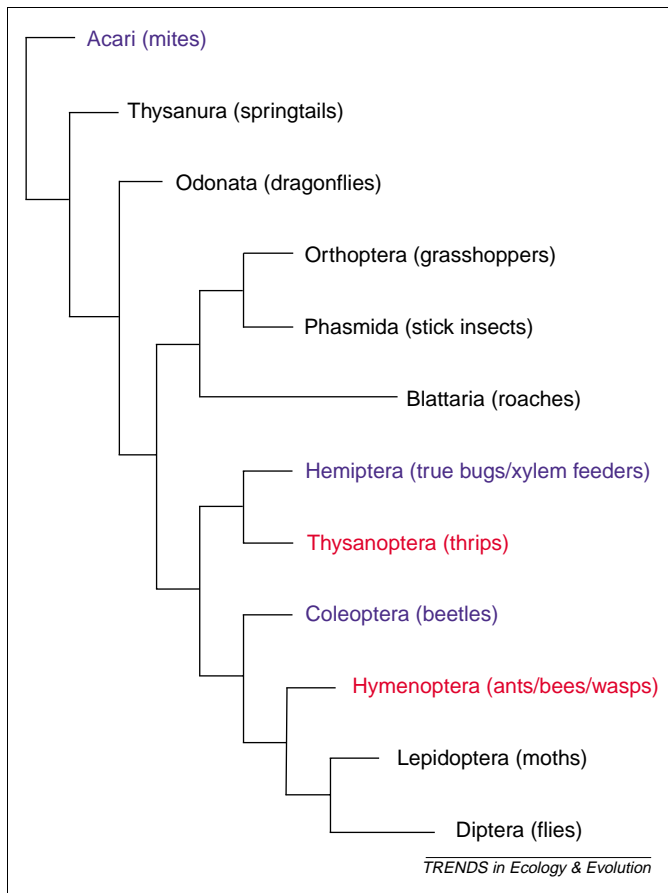


Figure 2. Known incidence of male haploidy (arrhenotoky) in arthropods. Purple, present in a few taxa; red, present in most taxa; black, not present. In addition, some insects show pseudo-arrhenotoky, whereby the paternal genome is lost during development. Ploidy data from [2], phylogeny after [22].

the components are derived from two distinct alleles. By contrast, dipteran *tra* shows essentially one allele, and functional proteins from this gene are encoded only by female-specific transcripts [8,9].

Beye *et al.* have determined the protein sequences of four *csd* alleles (there are probably ~20 such alleles in most populations, [10]). Allelic differences, in both length and sequence, are huge. Although the N-terminal regions were 70.3% identical across all four alleles, the arginine-serine domains and proline-rich regions within the C terminus were only 40.7% identical, with several insertion and/or deletions. The regions probably involved in specific protein-protein interactions also show the greatest degree of sequence variation among different alleles. This high degree of allelic variation is also seen in the self-incompatibility (*S*) loci in plants [11], suggesting that, similar to the *S* loci, *csd* is under strong selection to maintain polymorphism.

Comparative and population genetics

That *csd* in honeybees belongs to the same functional class (SR proteins) as the *tra* protein of *D. melanogaster* hints at a broad role for these proteins in sex determination, which can be explored using comparative sequence analyses. In flies studied to date, *tra* and *tra-2* (*transformer-2*) orthologues are inferred to be responsible for the female-specific splicing of the *doublesex* (*dsx*) gene [9,12] based on

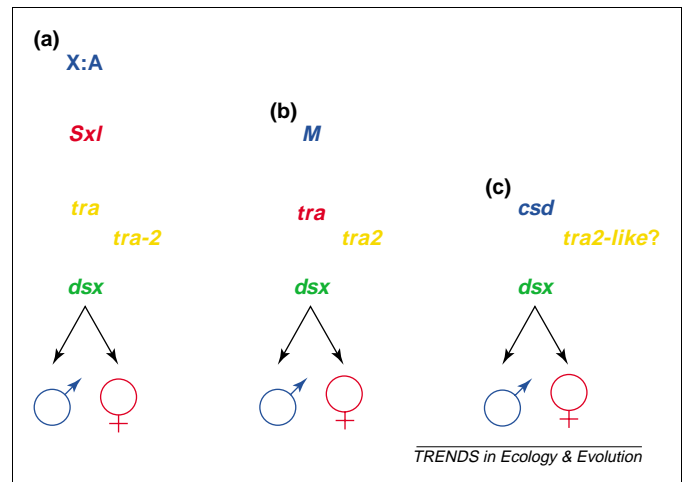


Figure 3. Models for somatic sex-determination pathways for *Drosophila melanogaster* (a), *Ceratitis capitata* (b) and the honeybee *Apis mellifera* (c). Blue text, primary signal; red, key gene; yellow, signal transduction; green, genetic doubleswitch. The bottom gene in all pathways, *dsx*, is conserved and is differentially spliced based on sex in all three species. The next gene in the *D. melanogaster* pathway, *tra*, has a functional orthologue in *C. capitata* and has some sequence similarity to the honeybee *csd*. In *D. melanogaster*, *tra2* is required for the transduction of the signal to *dsx*. *Tra2* is undescribed but believed to occur in *C. capitata*. The primary signal in each pathway is unique, ranging from the autosome:sex-chromosome (X:A) ratio in *D. melanogaster* to a dominant male determiner (M) in *C. capitata* and *csd* in honeybees. Abbreviations: *csd*, complimentary sex determiner; *dsx*, doublesex; *Sxl*, sexlethal; *tra*, transformer. (Based on [1,9,15].

functional data as well as characteristic binding domains within *dsx*. By contrast, *dsx* of the moth *Bombyx mori* has no *tra*-binding domains, implying that *tra* is not directly involved with the sex-specific splicing of *dsx* transcripts observed in this species [13] but not ruling out participation by other SR family members. To clarify the roles played by SR proteins, it will be of interest to see whether the sex-specifically spliced *dsx* orthologue in honeybees (identified by Becher and Beye as cited in [1]) is indeed regulated by *csd* in the same manner as *dsx* is by *tra/tra-2* in dipterans.

Evidence for the conservation of the basal sex-determination genes (*dsx*, *tra* and *tra2*) from several dipteran families suggests that sex-determination pathways evolved from the bottom up (Figure 3, [12,14]). If we compare the sex-determination pathways of those dipterans that have been elucidated in some detail (*Ceratitis capitata* [9] and *D. melanogaster* [15]) with that predicted for the honeybee, it appears that the latter pathway might be driven by only two steps (the basal gene *dsx*, the sex-specific expression of which is controlled by the primary signal *csd*) making this pathway the simplest yet discovered [1].

The molecular characterization of honeybee *csd* and *dsx* provides a starting point for understanding haplodiploidy in its diverse forms. Some haplodiploids, including many parasitic wasps and the fig wasps, have mating systems that promote extreme inbreeding (i.e. brother-sister matings are usual) [4]. Here, a single-locus complementation system as found in honeybees is predicted to be costly, because of the production of diploid males. Alternate mechanisms, including multiple complementary loci, any one of which might be heterozygous to spur female development [16], and genomic imprinting [17] have been proposed for inbreeding species, and recent evidence

implicates paternal-genome imprinting as a mechanism of sex determination in the parasitoid wasp *Nasonia vitripennis* [18]. Comparing these distinct mechanisms at the genetic level would provide fascinating insights into their evolution, and might help explain why advanced social Hymenoptera and other taxa depend upon an outbreeding mating system rather than imprinting or multiple complementary loci to avoid diploid males. These comparisons might also be extended to explore mechanisms of sex determination in species for which male haploidy results from loss of the paternal genome during development [2,19].

Finally, molecular characterization of *csd* sets the stage for population-genetic studies at this locus in bees and other arthropods. Characterization of functionally distinct alleles at this locus will help us to test models proposed to explain how social haplodiploids deal with a key genetic load (non-viable males resulting from homozygosity of *csd*) through the mitigating factors of multiple mating and the recognition and removal of diploid males [4]. Assuming orthologues are identified in other taxa, it should be possible to measure the sex-determination costs of small population sizes or bottlenecks throughout the Hymenoptera, and the effects of these costs on the balancing selection at *csd*. This will be especially relevant for understanding the population dynamics, and possible control strategies, for invasive species, such as fire ants (USA, [20]), bumblebees (Tasmania) or European wasps (New Zealand), which undergo population bottlenecks during colonization. As a second applied benefit, honeybee breeders and researchers endeavouring to develop genetically homogeneous lines should be able to avoid a very direct cost of inbreeding by choosing matings (even full-sibling matings) between individuals with distinct *csd* alleles. Screenings such as this could be used in the planning of germplasm banks (i.e. based on stored sperm, [21]) used to maintain unique honeybee lineages.

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Are men and women really so different?

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Distinct differences in the behaviour and preferences of men and women have conventionally been attributed to Trivers' powerful insights regarding the impact of parental investment on sexual selection and mating

systems. This has spawned a huge literature about the evolutionary significance of human sex differences. But are men and women really so different? An elegant new study shows that men and women are strikingly similar in their mate preferences. Have conventional models blinded us to the obvious, and precluded the posing of far more interesting questions?

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