5. Yoshida, S., and Pellman, D. (2008). Plugging the GAP between cell polarity and cell cycle. EMBO Rep. *9*, 39–41.

6. Long, A., Liti, G., Luptak, A., and Tenaillon, O. (2015). Elucidating the molecular architecture of adaptation via evolve and resequence experiments. Nat. Rev. Genet. *16*, 567–582.

7. Gresham, D., Desai, M.M., Tucker, C.M., Jenq, H.T., Pai, D.A., Ward, A., DeSevo, C.G., Botstein, D., and Dunham, M.J. (2008). The repertoire and dynamics of evolutionary adaptations to controlled nutrient-limited environments in yeast. PLoS Genet. *4*, e1000303.

8. Meyer, J.R., Dobias, D.T., Weitz, J.S., Barrick, J.E., Quick, R.T., and Lenski, R.E. (2012). Repeatability and contingency in the evolution of a key innovation in phage lambda. Science *335*, 428–432.

9. Szamecz, B., Boross, G., Kalapis, D., Kovacs, K., Fekete, G., Farkas, Z., Lazar, V., Hrtyan, M., Kemmeren, P., Groot Koerkamp, M.J., *et al*. (2014). The genomic landscape of compensatory evolution. PLoS Biol. *12*, e1001935.

10. Kvitek, D.J., and Sherlock, G. (2011). Reciprocal sign epistasis between frequently experimentally evolved adaptive mutations causes a rugged fitness landscape. PLoS Genet. *7*, e1002056.

11. Kryazhimskiy, S., Rice, D.P., Jerison, E.R., and Desai, M.M. (2014). Microbial evolution. Global epistasis makes adaptation predictable despite sequence-level stochasticity. Science *344*, 1519–1522.

12. Liu, G., Yong, M.Y., Yurieva, M., Srinivasan, K.G., Liu, J., Lim, J.S., Poidinger, M., Wright, G.D., Zolezzi, F., Choi, H., *et al*. (2015). Gene essentiality is a quantitative property linked to cellular evolvability. Cell *163*, 1388–1399.

13. Rancati, G., Pavelka, N., Fleharty, B., Noll, A., Trimble, R., Walton, K., Perera, A., Staehling-Hampton, K., Seidel, C.W., and Li, R. (2008). Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. Cell *135*, 879–893.

14. Bergmiller, T., Ackermann, M., and Silander, O.K. (2012). Patterns of evolutionary conservation of essential genes correlate with their compensability. PLoS Genet. *8*, e1002803.

15. Vernon, M., Lobachev, K., and Petes, T.D. (2008). High rates of "unselected" aneuploidy and chromosome rearrangements in tel1 mec1 haploid yeast strains. Genetics *179*, 237–247.

16. Nagasaki, A., de Hostos, E.L., and Uyeda, T.Q. (2002). Genetic and morphological evidence for two parallel pathways of cell-cycle-coupled cytokinesis in Dictyostelium. J. Cell Sci. *115*, 2241–2251.

17. Akiyoshi, B., and Gull, K. (2014). Discovery of unconventional kinetochores in kinetoplastids. Cell *156*, 1247–1258.

Sex Determination: Separate Sexes Are a Double Turnoff in Melons

Wen-Juan Ma and John R. Pannell*
Department of Ecology and Evolution, University of Lausanne, 1015 Lausanne, Switzerland
*Correspondence: john.pannell@unil.ch
http://dx.doi.org/10.1016/j.cub.2015.12.026

Flowers with only one sexual function typically result from the developmental suppression of the other. A recent study that shows how this is achieved has important implications for models of the evolution of separate sexes in plants.

Most plants are hermaphroditic, with all individuals possessing both male and female functions [1,2]. In the majority of these plants, male and female functions occur in the same flower — their flowers are so-called 'perfect'. Many angiosperms, however, have separate male or female flowers, either on the same individual (a state known as 'monoecy', with all individuals functionally hermaphroditic) or on different individuals (known as 'dioecy', with individual flowers being either fully male or female). In these species, unisexual flowers typically result from the suppression or sterilization of one of the two genders, with feminized and masculinized flowers lacking functional stamens and carpels, respectively (Figure 1). The extent to which the sterile gender is still represented by rudimentary and non-functional floral parts varies among species [3].

The question of why angiosperms "should ever have been rendered dioecious" puzzled Darwin [4] and has attracted a good deal of theoretical and empirical work ever since — what are the advantages of separate sexes, and under what conditions could mutations spread that suppress one or other of the two sexual functions, e.g., [5–7]? There appear to be plausible answers to these questions, with substantial empirical support (reviewed in [8]) supporting well-developed theory (reviewed in [9]). By contrast, the question of how, from a developmental genetics perspective, plants suppress carpels in some flowers and stamens in others, has remained a frustrated quest (but see [10]; reviewed in [3]). In a recent paper published in *Science*, Boualem *et al.* [11] describe elegant experiments with melons and cucumbers that reveals, for the first time, a network of genes involved in sex expression in monoecious plants with separate male and female flowers. Excitingly, although evidently not appreciated by the authors, their study also suggests how the full separation of the sexes might evolve along lines not invoked by classic evolutionary genetic models for dioecy in plants [7], but rather in ways similar to sex determination in well-characterised animals (reviewed in [12]).

The model for the gender development of flowers proposed by Boualem *et al.* [11] involves the interactive expression of

Figure 1. Examples of flowers with separate sexes.
(A,B) Male and a female flowers of the Mediterranean species *Ecballium elaterium*, respectively. (C) Monoecious inflorescence of *Sagittaria latifolia*, with a male flower held above three female flowers. (D) Monoecious inflorescence of *Mercurialis annua*, showing a developing fruit behind a cluster of male flowers in bud (one of which is open). All three species show within-species variation in their sexual system, with dioecious and monoecious populations in different parts of the species' ranges; they therefore illustrate possible intraspecific transitions between combined and separate sexes. Images (A), (B) and (C) courtesy of Marcel Dorken; image in (D) by John Baker.

genes at two different loci, each with direct effects on sex expression in individual flowers (Figure 2). The expression of a functional allele at locus *WIP1* suppresses carpel development, while that of a functional allele at locus *ACS-7* suppresses stamen development. These two loci are thus effectively female- and male-sterility loci, with the functional sterility allele showing dominant expression. Importantly, the two alleles also interact in their expression. Expression of *WIP1* not only suppresses carpel development, but also suppresses the expression of *ACS-7*, so that flowers expressing *WIP1* are female-sterile but produce functional stamens (i.e., they are male). Failure to express *WIP1* allows carpel development to proceed, simultaneously allowing *ACS-7* to suppress stamen development; thus, with *WIP1* switched off, flowers are fully female. Consistent with this model, genotypes that are homozygous for non-functional alleles at the *ACS-7* locus (i.e., *acs-7/acs-7* individuals) will produce male flowers when *WIP1* is turned on, but hermaphrodite flowers (with both stamens and carpels) when *WIP1* is turned off. Variation in the expression of *WIP1* among flowers of such individuals would be 'andromonoecious' (with both male and 'perfect' hermaphrodite flowers on the same plant).

This elegant model immediately prompts the question as to what determines whether *WIP1* is turned off or on. In fact, Boualem *et al.* [11] show that the expression of *WIP1* is regulated by an upstream locus, *ACS11* — when *ACS11* is active, *WIP1* is turned off (i.e., *ACS11* suppresses the expression of *WIP1*). But this just passes the buck. In a monoecious population, individuals produce both female and male flowers. In female flowers, *ACS11* is turned on, *WIP1* is suppressed (thus allowing carpel development), and the non-expression of *WIP1* allows *ACS-7* expression (thus suppressing stamen development). In male flowers, *ASC11* is turned off, thus allowing *WIP1* expression, which suppresses both the development of carpels and the expression of *ASC-7* (which then allows stamen development). So what decides whether *ASC11* is on or off in particular flowers?

The upstream regulation of *ASC11* is not known, but it is clear that, for functional monoecy, it must be switched on and off in different parts of the plant, destined to develop its female or male functions, respectively. Boualem *et al.* [11] note that the expression of *ACS11* in cucumber phloem points to ethylene as playing a role in determining the sex of flowers in particular parts of a plant, but the actual proximate signal still needs to be determined. Whatever the answer, it is important to remember that, in monoecious species, the sex is determined not by the segregation of a genetic polymorphism (as in dioecious species), but essentially in response to local cues to differentiating cells, which will differ between primordial destined to become male vs. female [3] (Figure 2). A transition from monoecy to dioecy thus ultimately requires the evolution of a genetic polymorphism for sex determination, so that some individuals only (or predominantly) produce male flowers and others only (or predominantly) produce female flowers [7].

The two-locus scheme proposed by Boualem *et al.* [11] immediately recalls models for the evolution of separate sexes (dioecy) in plants, which invoke the segregation of both male- and female-sterility mutations in populations [7]. Indeed, the authors suggest that their results point to a possible evolutionary route to dioecy, perhaps involving these two loci along lines predicted by theory. But the similarity between the two-locus scheme uncovered by the elegant work of Boualem *et al.* [11] and theory for the evolution of dioecy [7] is only superficial. Moreover, it may in fact point away from this now classic theoretical model, providing a potential counter example, rather than bolstering the model.

The empirical conclusions reached by Boualem *et al.* [11] are inconsistent with the classic two-locus model for the

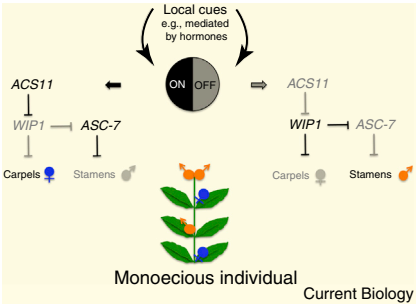

Figure 2. Cartoon of the gene network inferred by Boualem *et al*. [11] to be responsible for the development of male or female flowers in monoecious melons.
Black and grey text and symbols indicate genes and functions switched on and off, respectively. The 'flat-ended' arrows indicate suppression of activity. As detailed in the text, the model invokes three interacting loci, with gene *ASC11* suppressing the carpel suppressor *WIP1*, and *WIP1* also suppressing the stamen suppressor *ACS-7*, allowing flowers to develop carpels and to be fully female; expression of *WIP1* thus both prevents the production of carpels and suppresses *ACS-7*, allowing flowers to develop stamens and thus to be fully male. The suppressers at all three loci show dominant expression. In the absence of an expressed functional allele at *ACS11* (e.g., in *acs11/asc11* homozygotes), *WIP1* is always turned on and will result in the production of only male flowers, whereas, in the absence of a functional *ACS11*, *wip1/wip1* homozygotes would only produce female flowers. In monoecious individuals, an upstream regulatory cue (perhaps a hormone) turns *ASC11* on or off in different parts of the plant. In hypothetical dioecious populations, one could imagine alleles expressed at a single *trans*-acting locus acting to turn *ASC11* on or off.

evolution of dioecy in a number of interesting ways. First, the most likely scenario modelled [7] involves the invasion of a recessive male-sterility mutation rendering the population gynodioecious (females and hermaphrodites in the population), followed by the invasion of one or more female-sterility mutations with dominant expression; this scenario would yield a dioecious population with XY sex determination. Yet both the male- and the female-suppressing alleles revealed in Boualem *et al.*'s [11] study show dominant expression.

Second, the two- (or, if we include the upstream regulator, the three-) locus network discovered by Boualem *et al.* [11] quickly collapses to a single-locus model when construed as a segregating polymorphism, as required for dioecy. In a satisfying conclusion to their study, Boualem *et al.* [11] crossed female and male lines constructed using a combination of loss-of-function mutations at both the *WIP1* locus and the upstream locus *ACS11*. Specifically, a female, constructed to be homozygous for *wip1* and *acs11*, was crossed with a male, constructed to be homozygous for *acs11* but heterozygous for *wip1* (i.e., *wip1/WIP1*). As expected, the progeny segregated for males (genotype *wip1/WIP1*) and females (genotype *wip1/wip1*) in a 1:1 ratio. In other words, the population generated by this cross was fully dioecious, with sex determined by an effective XY system (and the chromosomes harbouring the *wip1* and *WIP1* alleles acting as the X and Y chromosomes, respectively). Importantly, the dioecious population artificially created by Boualem *et al.* [11] ended up having a single locus sex-determination system, because only one locus was segregating for different alleles (the *WIP1* locus). This is not the two-locus model invoked by models for the evolution of dioecy from hermaphroditism [7].

The fundamental difference between the scheme proposed by Boualem *et al.* [11] and the two-locus model for dioecy [7] is that the former invokes epistasis between two (or more loci), whereas the latter assumes independent gene action at male- and female-sterility loci. This is important, and potentially very revealing. It indicates that dioecy could evolve from functional hermaphroditism through the invasion into the population of a single mutation altering expression of a single gene acting upstream of a network of interacting developmental genes, such as the one discovered by Boualem *et al.* [11], and such as those that have been deciphered for sex determination in animal systems, e.g., *SRY* and *DMRT1*, which are necessary for male development in metazoans (reviewed in [13]).

Recent work on the evolution of sex determination and sex chromosomes in plants has revealed patterns and processes that are strikingly similar to those known in animal systems (reviewed in [14]), including the suppression of recombination [15], the degeneration of Y chromosomes (or W chromosomes in ZW systems; e.g., [16]), and the evolution of the modification of gene expression on sex chromosomes to compensate for gene loss on one of the chromosomes ('dosage compensation') [17,18]. The study by Boualem *et al.* [11] now shows that, just as in animals, plant sex determination could involve a single locus of sex determination as the switch gene for a complex network of interacting genes that influence each other's expression. It will be interesting to discover what triggers sex determination in dioecious species related to the melons and cucumbers studied by Boualem *et al.*, to see whether the same genes are involved downstream, and to understand what evolutionary path was followed to get there.

REFERENCES

1. Renner, S.S. (2014). The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. Am. J. Bot. *101*, 1588–1596.
2. Barrett, S.C.H. (2002). The evolution of plant sexual diversity. Nat. Rev. Genet. *3*, 274–284.
3. Diggle, P.K., Di Stilio, V.S., Gschwend, A.R., Golenberg, E.M., Moore, R.C., Russell, J.R.W., and Sinclair, J.P. (2011). Multiple developmental processes underlie sex differentiation in angiosperms. Trends Genet. *27*, 368–376.
4. Darwin, C. (1877). The Different Forms of Flowers on Plants of the Same Species (New York: Appleton).
5. Lloyd, D.G. (1982). Selection of combined versus separate sexes in seed plants. Am. Nat. *120*, 571–585.
6. Lloyd, D.G. (1975). The maintenance of gynodioecy and androdioecy in angiosperms. Genetica *45*, 325–339.
7. Charlesworth, D., and Charlesworth, B. (1978). A model for the evolution of dioecy and gynodioecy. Am. Nat. *112*, 975–997.
8. Webb, C.J. (1999). Empirical studies: evolution and maintenance of dimorphic breeding systems. In Gender and Sexual Dimorphism in Flowering Plants, M.A. Geber, T.E. Dawson, and L.F. Delph, eds. (Berlin Heidelberg: Springer), pp. 61–95.
9. Charlesworth, D. (1999). Theories of the evolution of dioecy. In Gender and Sexual Dimorphism in Flowering Plants, M.A. Geber, T.E. Dawson, and L.F. Delph, eds. (Heidelberg: Springer), pp. 33–60.
10. LaFlamme, B. (2014). Persimmon sex determination. Nat. Genet. *46*, 1257.
11. Boualem, A., Troadec, C., Camps, C., Lemhemdi, A., Morin, H., Sari, M.A., Fraenkel-Zagouri, R., Kovalski, I., Dogimont, C., Perl-Treves, R., *et al.* (2015). A cucurbit androecy gene reveals how unisexual flowers develop and dioecy emerges. Science *350*, 688–691.

12. Beukeboom, L.W., and Perrin, N. (2014). The Evolution of Sex Determination (Oxford: Oxford University Press).

13. Graves, J.A.M. (2013). How to evolve new sex determining genes. Dev. Dyn. *242*, 354–359.

14. Charlesworth, D. (2015). Plant contributions to our understanding of sex chromosome evolution. New Phytol. *208*, 52–65.

15. Charlesworth, D. (2013). Plant sex chromosome evolution. J. Exp. Bot. *64*, 405–420.

16. Marais, G.A.B., Nicolas, M., Bergero, R., Chambrier, P., Kejnovsky, E., Moneger, F., Hobza, R., Widmer, A., and Charlesworth, D. (2008). Evidence for degeneration of the Y chromosome in the dioecious plant *Silene latifolia*. Curr. Biol. *18*, 545–549.

17. Bergero, R., Qui, S., and Charlesworth, D. (2015). Gene loss from a plant sex chromosome system. Curr. Biol. *25*, 1234–1240.

18. Papadopulos, A.S.T., Chester, M., Ridout, K., and Filatov, D.A. (2015). Rapid Y degeneration and dosage compensation in plant sex chromosomes. Proc. Natl. Acad. Sci. USA *112*, 13021–13026.

Evolution: Causality and the Origin of Parasitism

Jan Janouskovec[1] and Patrick J. Keeling[2]
[1]Department of Biology, San Diego State University, San Diego, CA, USA
[2]Department of Botany, University of British Columbia, Vancouver, BC, Canada
Correspondence: janjan.cz@gmail.com (J.J.), pkeeling@mail.ubc.ca (P.J.K.)
http://dx.doi.org/10.1016/j.cub.2015.12.057

The first comparison of parasitic trypanosomatids to their free-living relatives reveals that some characteristics once linked to parasitism actually predate it. Parallel comparisons of other parasites suggest we need to rethink the drivers and consequences of the parasitic lifestyle.

Evolution works in mysterious ways, and one problem that biologists must confront is the difficulty in establishing causality. Understanding a biological transition means understanding not only what events took place, but also their order, and which events precipitated others. But we often predict order and causality by pure intuition; we observe characteristics in some organism or lineage that operate under a current set of conditions and then try to imagine a progression of events that gave rise to these characteristics. Often, however, we put the causal cart before the horse because we lack critical information, typically relating to the nature of the ancestral and intermediate states in this transition. Our understanding of the evolution of parasites and pathogens illustrates this problem all too well. While we have established concepts like the Bradford Hill criteria and Koch's postulates to at least guide our thinking about disease causation [1], understanding causal factors in the emergence of pathogens at evolutionary time scales has been more *ad hoc*. One reason for this is because we focus on the final state of this transition, the pathogen, and lack almost any information on the initial state, represented today by the pathogen's free-living relatives. In a recent paper in *Current Biology*, Jackson *et al.* [2] approach the origin of trypanosomatids, an important group of kinetoplastid parasites including *Trypanosoma* and *Leishmania*, by characterizing the genome of a free-living relative, the bacterial grazer *Bodo saltans*. By looking from the outside in, Jackson *et al.* [2] describe a number of noteworthy patterns about the kinetoplastid transition to parasitism that upend more than a few ideas about what led the kinetoplastids to become parasites.

Parasites abound with specialized characteristics associated with disease, and these are commonly interpreted as adaptations to the parasitic lifestyle. This may be true, but it may also be a reflection of our tendency to associate current specialization with the immediate biological context, and not with the context in which it first arose.

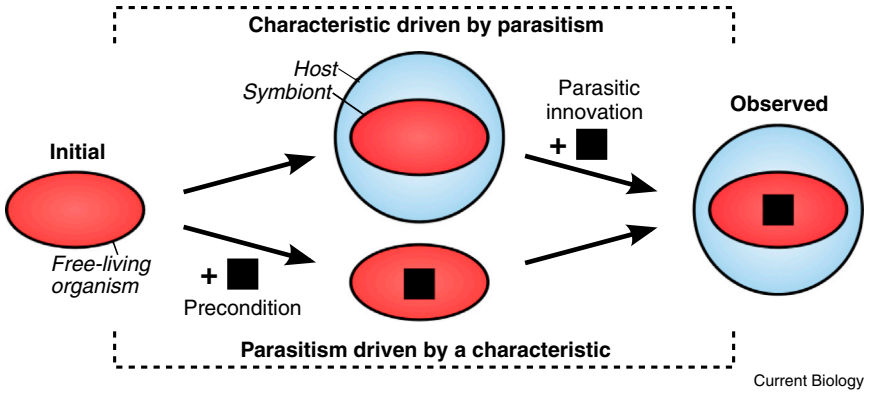

Figure 1. Models for the transition of free-living organisms to parasites, and their character states.
Two alternative ways to interpret the characteristics of parasites and how they relate to the origin of parasitism. On the top, a characteristic evolves as an adaptation to the existence of a parasitic interaction. On the bottom, the characteristic evolves in a free-living organism, but its existence makes the transition to parasitic life more likely, so it preconditions the lineage to a parasitic transition.

