From common gardens to candidate genes: an elegant case of homoploid hybrid speciation

Homoploid hybrid speciation (HHS), where genetic contributions from two species combine to produce a third species without a change in ploidy, remains an intriguing and enigmatic evolutionary process. Genomic studies in the past decade have shown that introgression and admixture between species is common, and yet well documented cases of HHS remain rare (Schumer et al., 2014; Marques et al., 2019). This strikes at a key feature of HHS; it is a far higher bar to prove that hybridization was critical for speciation than it is to show that introgression occurred. The former requires us to know how reproductive isolation (RI) is achieved, identify hybrid ancestry, and definitively link hybrid ancestry with reproductive isolation. With this in mind, the work by Wang et al. (2020) fully documenting a case of HHS is particularly impressive in terms of both the scope and clarity of the results.

Wang et al. (2020) focus on the three species in the genus *Ostryopsis*: *Ostryopsis nobilis*, *Ostryopsis davidiana*, and the homoploid hybrid species *Ostryopsis intermedia* (Tian et al., 2010). They first characterized RI, including using an eight-year common garden study as well as *in vitro* and vivo experiments, and found a particularly elegant pattern; the parental species are isolated by habitat type (soil iron content) as well as flowering time, while the hybrid species has a mix of parental traits, thereby retaining isolation to both parents by one mechanism each (Figure 1). This fulfills the first requirement of HHS. To fulfill the second requirement, Wang et al. (2020) assembled full genomes for all three species involved and re-sequenced populations of each. They used several different analyses to prove hybrid ancestry and show that the genome is 78% from *O. davidiana* and 22% from *O. nobilis*.

The last requirement of HHS is demonstrating that RI is due to hybrid ancestry. Wang et al. (2020) leverage their genomic data to look for positively selected genes shared by *O. intermedia* and each of its parental species in turn. For example, both *O. intermedia* and *O. nobilis* have higher iron tolerance than *O. davidiana*, so they group *O. intermedia* and *O. nobilis* together and compare them with *O. davidiana* using the Hudson-Kreitman-Aguadé (HKA) test to identify positively selected genes (Hudson et al., 1987). Since *O. intermedia* and *O. davidiana* share an early flowering time, they used the opposite grouping to identify positively selected genes involved in flowering time differences. This approach identified several excellent candidate genes; *ZIP5* and *FRO4* for iron tolerance and *LHY*, *PIE1*, and *FPA* for flowering time. The authors transgenically introduce alleles from three of these genes into *Arabidopsis* and show predictable phenotypic effects. Taken together, this suggests that *O. intermedia* speciated by taking iron tolerance alleles from *O. nobilis* and early flowering alleles

from *O. davidiana*. This is the first paper to link HHS to candidate genes, and it will be particularly interesting to see if the approach of using the HKA test is effective in discovering candidate genes in other hybrid species.

The authors propose that the sorting of prezygotic RI alleles, like that occurring in *O. intermedia*, is a new model of HHS. This is in contrast with most recent models, which focused on the sorting of intrinsic RI loci, such as Bateson-Dobzhansky-Muller (BDM) incompatibilities (Schumer et al., 2015; Blanckaert and Bank 2018). By using prezygotic RI loci, this means that HHS can occur with only two loci, rather than four, but in some ways it seems to be a less generic model. While BDMs can be any negative interactions between loci, the prezygotic isolating RI loci described here require the interaction with an environmental variable (i.e., soil iron content) or involve mate choice. The evolution of BDMs is likely inevitable between any two isolated populations, but prezygotic RIs are not. On the other hand, environmental adaptation and flowering time differences are ubiquitous, so the raw materials for HHS are certainly present.

One further possible extension of the prezygotic sorting model is incorporating greater interactions with the parental species. In the current simulations, a hybrid population is defined and gene flow with the parental species is cut off after the BC1 generation, preventing the possibility of a hybrid zone. While this simplifies the modeling, in many hybrid populations gene flow with parents continues. Similarly, it remains to be seen how varying the strength and genetic architecture of RI genes affects the probability of HHS. Current simulations use unlinked relatively high-strength RI loci, but is the model still effective when RI is highly polygenic and linked?

The ultimate importance of this model of HHS will be shown if other hybrid species match its predictions of sorting prezygotic RI loci. This will be a challenge in animal systems where the genetic mechanisms of mate choice are much less understood than, for example, the control of flowering time is in plants. Wang et al. (2020) show some interesting patterns analyzing the genomes of a hybrid species of Darwin's finches, but more work is needed to validate candidate genes (Lamichhaney et al., 2018). For example, are candidate genes related to vision actually involved in visual mate choice?

In summary, Wang et al. (2020) have presented an amazingly complete example of HHS and it would be well advised for others in the field to aim for their standard. Although much of
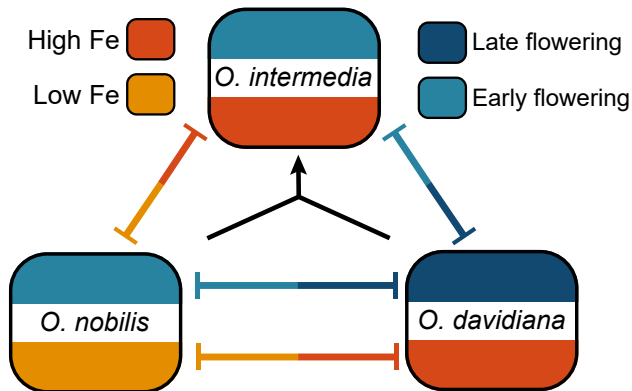
Figure 1. A schematic diagram of RI in *O. davidiana*, *O. nobilis*, and the hybrid species *O. intermedia*.
Colors on top of species names represent flowering time and colors below names represent soil iron tolerance. Colored bars between species represent RI traits.

the work presented involved cutting-edge genomics and transgenics, it is all underpinned by classic common garden and field work that quantified RI. It is this combination of old and new techniques that is the paper's strength.

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