

1 Distyly supergenes as a model to understand the evolution of genetic architecture.

2 Genetic architecture, the relative positions of genes across the genome, can appear random with
3 genes scattered without regard to function, but evidence suggests that it is shaped by evolution. In
4 particular, the evolutionary trajectories of functional traits can both be influenced by and influence
5 their underlying genetic architecture. Where multiple loci contribute to local adaptation, coadapted
6 gene complexes can form that consist of combinations of alleles whose interactions have been
7 shaped by selection to optimize trait expression (Prakash and Lewontin, 1968; Allard et al., 1972).

8 Coadapted gene complexes, however, are vulnerable to disruption by recombination following gene
9 flow from outside the zone of local adaptation if different zones have distinct sets of alleles. The
10 power of recombination to disrupt allelic combinations is reduced if loci are situated in close physical
11 proximity to each other in the genome. Models of the evolution of genetic architecture show that
12 divergent gene clusters can emerge under selection with gene movement and turnover across the
13 genome (Yeaman, 2013; Lindke and Buerkle, 2015). A growing body of empirical evidence of
14 clustered genomic architectures underlying adaptive traits supports these ideas (Table 1). The extent
15 to which adaptation avails of pre-existing genetic architecture or whether genetic architecture
16 evolves in response to selective pressure is an open question, however. Answers will come from
17 systems where the genetic architecture underlying an adaptive trait has important consequences for
18 expression and function.

19 The supergenes that control expression of distyly are a compelling example of the importance of
20 genetic architecture for the expression and evolution of an adaptive trait. Distyly is a floral
21 polymorphism with two floral morphs. Tristyly species with three floral morphs are also known
22 but the extra complexity of tristylous systems is not considered further in this essay. In distyly, the
23 stigma and anthers are separated by length differences within flowers but the length differences are
24 switched in the other floral morph. These floral organ length differences favour reciprocal pollen
25 transfer between morphs, thereby promoting disassortative pollen transfer and reducing pollen
26 wastage (Lloyd and Webb, 1992). In addition to the minimum requirement of different style and
27 filament lengths, or reciprocal herkogamy, in each floral morph, the distylous flowers often also
28 show intra-morph-incompatibility and differing ancillary pollen and stigma characteristics (Lloyd and
29 Webb, 1992).

30 Classical genetic studies across several species show distyly to be under the simple genetic control of
31 a single diallelic locus (Charlesworth and Charlesworth, 1979; Barrett and Shore, 2008). The two
32 alleles interact in a dominant-recessive manner so that heterozygous individuals express one flower
33 morph and recessive allele homozygotes express the other morph. This mode of inheritance insures
34 that inter-morph crosses will generate equal morph proportions of progeny as favoured by negative
35 frequency-dependent selection to maximise mate availability. This simple genetic basis disguises an
36 interesting genetic conundrum: how can a single locus be responsible for the multiple distinct floral
37 traits that typically distinguish the different floral morphs? This is solved by the presence of a
38 supergene consisting of multiple highly-linked genes with morph specific alleles enabling the
39 multiple traits comprising each floral morph to be inherited as a single unit (Charlesworth 2016).
40 Large non-recombining regions at supergenes have been identified in some distylous species (> 610
41 kb in *F. esculentum*; Yasui et al., 2012, 278 kb in *P. vulgaris*; Li et al., 2016).

42 Far from being an evolutionary curiosity, the phylogenetic distribution of distyly suggests as many as
43 28 independent evolutionary origins. Corroborating this, different candidate genes for distyly have
44 been identified in different plant families. Five genes: *GLO^T*, *CYP^T*, *CCM^T*, *PUM^T* and *KFB^T*, have been
45 identified at the newly sequenced *Primula vulgaris* S locus (Li et al., 2016; see also Nowak et al.,
46 2015; Huu et al. 2016). Of these genes, *GLO^T* *CYP^T* have been found to control anther position and
47 style length, respectively. Other candidate genes have been identified in distylous species from other
48 plant families: *TSS1* in *Linum grandiflorum* (Linaceae; Ushijima et al., 2012), *S-ELF3* in *Fagopyrum*
49 *esculentum* (Polygonaceae; Yasui et al., 2012), and *TsSPH1* in *Turnera sublata* (Turneraceae; Labonne
50 and Shore, 2011).

51 Genetic architecture therefore plays a central role in the expression and function of distyly, but how
52 does it evolve? Two alternative evolutionary scenarios are possible. Scenario 1: A pre-existing
53 genetic configuration facilitates the evolution of distyly. The minimum requirements for the
54 establishment of a distyly supergene involve the chance co-location of two loci influencing style and
55 filament length respectively. Selection against recombination between this pair of loci could lead to
56 a supergene zone of low recombination, “trapping” neighbouring loci that could then be recruited to
57 further refine floral form. Scenario 2: Genetic architecture evolves subsequent to the establishment
58 of distyly. In this scenario, the recruitment of genes by translocation into the supergene zone allows
59 the evolution of increasingly complex floral polymorphisms to maximise the efficiency of cross-
60 pollination. Empirical evidence supporting scenario 1 comes from the analogous situation of the
61 gradual expansion of sex determining regions and sex chromosomes through the suppression of
62 recombination (Charlesworth 2016) and co-segregation of functionally unrelated traits such as the
63 *hose in hose* and *oakleaf* mutants reported for *P. vulgaris* (Li et al., 2016). However, as floral
64 polymorphisms become increasingly elaborate, it seems unlikely that all the functional loci involved
65 owe their presence to chance juxtaposition prior to the evolution of distyly.

66 The near future of heterostyly research will look beyond identifying individual loci underlying
67 different floral morph phenotypes in individual species and their close relatives and will move
68 towards characterizing the entire supergene structure of distylous species from multiple
69 independent origins. Within-family analyses will be able to track the evolution of supergene
70 structure itself by determining the order in which genes are recruited to distyly function (Figure 1).
71 Scenario 1 will be supported if the S locus genes contributing to distyly are also physically close in
72 outgroup taxa that diverged before the origin of distyly. Scenario 2 will be supported if more derived
73 family members show progressively more S locus genes than more basal members and that these
74 genes were recruited to distyly function through translocation.

75 Plant mating systems frequently transition from outcrossing to selfing, which in this case involves
76 reversion of distyly to homostyly (Barrett and Shore, 2005). Several examples of loss of distyly will
77 likely be present in large taxonomic samples (Sakai and Wright, 2008; McDill et al., 2009). Study of
78 these transitions and the subsequent decay of supergenes will provide insight into the selective
79 forces that once maintained supergene architecture. Loss of gene function, resumption of
80 recombination, and supergene restructuring are some of the potential outcomes following loss of
81 distyly. But which of these processes is the typical initial trigger for loss of distyly?

82 The study of distyly across multiple evolutionary scales will be invaluable to our understanding of the
83 evolution of co-adapted genomic islands more generally. Independently evolved distyly systems

84 represent independent evolutionary experiments that can be compared and contrasted. Large
85 genera with a mix of homostylous and distylous species such as *Psychotria* (Rubiaceae) might be
86 useful for such studies (Sakai and Wright, 2008), while herbaceous genera such as *Primula* or
87 *Turnera* are more experimentally tractable. Between-family comparisons would provide insights into
88 general mechanisms by which recombination is locally suppressed and allelic dominance achieved.
89 Intriguingly, recent findings in *Turnera* and *Primula* indicate that distyly genes are hemizygous with
90 different morph types resulting from the presence or absence of a single supergene haplotype
91 (Labonne and Shore, 2011; Nowak et al., 2015; Li et al., 2016). A single morph-specific supergene
92 haplotype solves both the problem of recombination between different morph supergenes and the
93 control of dominance interactions; the dominant phenotype being conferred by supergene
94 presence. It remains to be seen how general a solution to distyly genetic architecture this might be.

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144 Table 1. Adaptions influenced by their underlying genetic architecture.

Genetic architecture	Example
Functionally linked gene cluster	Some diterpenoid and triterpenoid biosynthetic pathways in rice (Swaminathan et al. 2009)
Chromosomal rearrangement	Ecologically distinct parent and hybrid species in sunflowers (Reiseberg et al., 1995)
Chromosomal inversion	Life history and ecological differences in monkeyflowers (Twyford and Friedman, 2015)
Sex determining chromosome/region	Dioecy, separate male and female individuals in campion (Bergero and Charlesworth, 2011)
Centromeric region	Sexual spores containing both mating types in anther smut fungus (Hood and Antonovics, 2004)

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147 Figure 1. Distyly evolution involves the recruitment of linked genetic loci to a non-recombining
 148 supergene. Legend. Black lines depict evolutionary diversification within a family resulting in species
 149 A to G at the tips of the phylogenetic tree. Coloured images represent floral structure in each species
 150 with blue representing petals, yellows and oranges representing stamens and pollen, and greens
 151 representing pistils. Letters along the phylogenetic tree branches represent genetic loci involved in
 152 the distyly phenotype. Species A shows an ancestral homostylous phenotype. Species B shows an
 153 approach herkogamy polymorphism with differences in female (*F*) organ length. Species C shows
 154 with additional reciprocal changes in male (*M*) organ length. Additional elaborations to distyly are
 155 then possible such as self-morph incompatibility (species D; gene *I*), pollen size (species E; gene *P*),
 156 and floral organ repositioning (species F; gene *R*). These traits could evolve in any order but their
 157 controlling loci must be linked to the supergene to be associated with distyly. Species G indicates
 158 that evolution is not necessarily directional and distyly can also be lost.

