Genomic studies of adaptive evolution in outcrossing Arabidopsis species

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Abstract

Large-scale population genomic approaches have very recently been fruitfully applied to the Arabidopsis relatives *Arabidopsis halleri*, *A. lyrata* and especially *A. arenosa*. In contrast to *A. thaliana*, these species are obligately outcrossing and thus the footprints of natural selection are more straightforward to detect. Furthermore, both theoretical and empirical studies indicate that outcrossers are better able to evolve in response to selection pressure. As a result, recent work in these species serves as a paradigm of successful population genomic studies of adaptation both to environmental as well as intracellular challenges.

Introduction

The advent of population-scale genomic approaches is revolutionizing our ability to study evolution and adaptation in wild species. The Arabidopsis genus, with several outcrossing species, small genomes and a diversity of adaptations, is increasingly being leveraged to understand adaptation as well as the influence of processes like repeated evolution, hybridization and adaptive gene flow.

Recent genus-wide resequencing showed that hybridization has likely played an important role in the history of the outcrossing *Arabidopsis* species and can to some extent blur the boundaries between taxa [1]. Hybridization is particularly extensive between tetraploid lineages of *A. lyrata* and *A. arenosa*, which show no ongoing hybridization at the diploid level, highlighting that genome duplication can cause species boundaries to become more porous again after a period of genetic isolation among the diploid progenitors [2]. Nevertheless, the large groupings corresponding to the named species *A. arenosa*, *A. lyrata* and *A. halleri* can be clearly recognized from genomic data. These three species have adapted to a wide range of habitats (e.g. Fig. 1) and represent tremendous opportunities for studying adaptive evolution. Importantly, all of these species are obligate outcrossers, which means that compared to *A. thaliana*, populations of these species will show stronger local adaptation and the signals will be easier to detect (Box 1).

Arabidopsis arenosa

Arabidopsis arenosa has recently been the subject of a concentrated set of explicitly genome-wide studies of adaptive evolution. These provide case studies for how population genomic approaches can be fruitfully applied to diverse biological questions,

from detailed understanding of range-wide demography to novel insights to adaptation and evolution.

Demography. To contextualize studies of adaptation, it is important to understand the demographic history of the populations under study. From previous biogeographic studies we know that *A. arenosa* is found throughout Europe, with diploids found in the Balkans and along the Baltic coast, and tetraploids through the Alps, Carpathians, and Central and Northern Europe [3,4], with a center of diversity in the Western/Northern Carpathians of Slovakia [4].

Increasingly, large-scale genomic approaches are being applied to understand species histories and demographic dynamics. A particularly powerful tool for demographic analyses is reduced representation sequencing (RADseq) which allows cost-effective sampling of thousands of individuals [5,6]. RADseq approaches have recently been applied in *A. arenosa* to better understand its history and patterns of genetic differentiation across the landscape. A recent study of diploid *A. arenosa* showed that genetic variation sorts into four clearly distinct gene pools, with a fifth showing a hybrid origin [3]. Some populations show striking ecological differentiation with only minimal genetic differentiation, making them promising models for further investigation of the genetic basis of local adaptation, since signals of selection do not need to rise above strong noise [3]. A recent analysis leveraging RADseq, POOLseq of whole genomes, and whole genome resequencing of barcoded individuals, placed the origin of the autotetraploid *A. arenosa* lineage within a single diploid gene pool from the western Carpathian Mountains 15,000-19,000 generations ago [7]. The tetraploids subsequently split into five genetically distinct lineages that colonized different geographic regions and

habitats and some populations subsequently experienced bidirectional gene flow with geographically proximal diploids [7]. Demographic studies are summarized in Fig. 2.

Dealing with whole genome duplication. Polyploids occur in all eukaryotic lineages, but are particularly common in plants [8]. However, recently formed polyploids face substantial challenges, particularly with the regular segregation of the additional chromosomes [9-11]. How early polyploid lineages can evolve to stabilize meiotic chromosome segregation (and any other problems associated with genome duplication) has remained largely mysterious. But recent genome scans in *A. arenosa* have shed some light on this. The autotetraploid lineage of *A. arenosa* arose recently enough to remain closely related to its diploid ancestor, but is sufficiently ancient that evolution has had sufficient time to stabilize its meiosis, and presumably other problems associated with WGD [12**]. This system thus provides a rare opportunity to study the early evolutionary stabilization of polyploid lineages and how polyploids might adapt to their novel genomic context.

Two population level resequencing scans in *A. arenosa* yielded a set of genes with clear signatures of selection in tetraploids and strong differentiation between diploids and tetraploids [12**,13]. Consistent with meiotic chromosome segregation being a major challenge for polyploids, among the 19 genes with the strongest evidence for selection genome-wide are eight core structural meiosis proteins that have essential roles in recombination, chromosome pairing, and segregation [12**]. Other genes with strong signatures of selection included several chromatin remodelers, genes implicated in cell size regulation, cell cycle regulators, and core transcriptional regulators. The role these genes might play in polyploid evolution remains to be tested, but these results highlight

how the hints provided by genome scans open the door to generation of new testable hypotheses.

Interestingly, in the *A. arenosa* tetraploid lineage, selection targeted genes that are also implicated in other polyploid contexts: several genes important for chromosome cohesion and segregation were also identified in a yeast screen for genes necessary for survival of polyploid but not diploid cells [14], and several have been implicated in genome instability in polyploid and derivative aneuploid cells in human cancers (reviewed in [15]). This suggests that there are consistencies in the challenges faced by polyploid cells even across kingdoms, and that what we learn about the evolution of chromosome segregation in a polyploid context from *A. arenosa* can serve to inform a very wide set of research directions[15].

Meiosis gene evolution in diploids. A recent investigation of selection on meiosis genes relative to genome-wide patterns showed that several meiosis genes under selection in tetraploids were also under selection independently in a diploid lineage [16], Though the reasons for this are as yet unclear. However, this study raised a new hypothesis: This diploid lineage originates from the Pannonian basin in Hungary, a warmer environment than that of the sites the mountain plants from the Carpathians it was compared to. Together with abundant evidence that meiotic structures are particularly sensitive to high temperature [17], this raised the possibility that this meiotic adaptation may have something to do with ambient temperature.

Ruderal habitat adaptation. As seen in many of its relatives, *A. arenosa* populations also vary in respect to their flowering time. In *A. arenosa* this variation is associated with habitat divergence [18,19]: mountain populations found in usually sheltered sites are

perennial and late flowering in laboratory conditions, and require vernalization (prolonged cold treatment) to flower early, while plants from sites on railways are consistently early and perpetually flowering in the lab. Transcriptome analysis and genome scans for selection were combined to understand the evolution of these "weedy" traits in railway *A. arenosa* populations. The whole-genome transcriptome analysis showed that early flowering in railway populations is associated with loss of expression of the vernalization-sensitive floral repressor FLC, and an associated abrogation of the entire vernalization response [18]. This parallels results from another perennial outcrosser, Arabis alpina, where variation in activity of an FLC homolog, PEP1 is causal for a similar shift from vernalization responsive late flowering, to rapid cycling and perpetual flowering [20]. However, the transcriptome analysis of flowering highlights a particular utility of this approach - in the analysis, it became evident that in addition to flowering related genes, cold-responsive genes as well as heat-shock factors that are normally inducible became constitutive in the railway lineage. This prompted the authors to test for elevated heat and cold stress tolerance, and indeed, railway plants showed constitutive heat and cold tolerance, while in mountain plants, these responses need to be induced by prior exposures [18]. Genome scans for selection in these plants complemented these results and provided candidate genes for the upregulation of these genes: LHY, a circadian clock gene that is a known inducer of cold-responsive genes [21,22], and LARP1, an mRNA splicing factor important in heat response [23]. Whether these genes are causal remains to be tested, but this nevertheless provides a case study for how transcriptome analyses and genome scans can be effectively combined.

Substrate adaptation. Like its outcrossing Arabidopsis relatives, *A. arenosa* is found on a variety of particularly challenging substrates, from zinc mine tailings [24,25] to beaches [3], to serpentine barrens [26]. Serpentine barrens are particularly harsh and represent a

perfect storm of low mineral nutrient, drought prone, and toxic metal rich conditions and thus offer a powerful model of multi-challenge adaptation. A genome resequencing scan comparing closely related serpentine and non-serpentine *A. arenosa* populations found evidence for highly localized selective sweeps that point to a polygenic, multitrait basis for serpentine adaptation [27**]. Comparing results from that study to a previous study of independent serpentine colonizations in *A. lyrata* [28**] the highest levels of differentiation were found in 11 of the same genes, but the alleles selected were not the same, providing candidate genes for convergent evolution. In addition, it appears that other selected alleles were introgressed geographically proximal *A. lyrata* populations, providing a clear example of transpecies adaptive gene flow [27**].

Narrow peaks – the joy of outcrossers? One thing that has been striking in the genomic analyses of selection in *A. arenosa* is how narrow the peaks of selection are, which makes identification of candidate genes unambiguous in most cases. While the causes for this are not entirely clear, a major factor is likely mating system. Unlike *A. thaliana, A. arenosa* is an obligate outcrosser. Large effective population sizes and high effective recombination rates make selection powerful in outcrossers, and allow randomization of linked polymorphisms. This likely helps explain the narrow and strong selection peaks, and highlights how much more powerful outcrossers are as models for evolution and adaptation (see Box 1).

Arabidopsis lyrata and A. halleri

The other two major outcrossing species in the Arabidopsis genus are *A. lyrata* and *A. halleri. A. lyrata* has a published high-quality reference genome [29] and was the subject of what may perhaps be called the first truly modern population genomic study in the Brassicaceae [28]. This study investigated the basis of adaptation to serpentine soils by

resequencing pooled samples of four populations, two of which represented independent serpentine colonizations. Using a straightforward differentiation metric-based approach, an array of sensible candidate loci were identified as potentially mediating serpentine tolerance. To some degree these overlap with those found in *A. arenosa* [27**] suggesting selection acted on the same target loci in both species independently, highlighting their likely central role in adaptation to this high-stress habitat.

Arabidopsis halleri is best known as a workhorse for the study of metal hyperaccumulation [30-32], but as yet population genomic approaches have not been applied to understanding this extremophile adaptation. However, two interesting population genomic studies recently outlined the genomic basis for altitudinal adaptation in *A. halleri*. Fischer et al. [33] applied Pool-Seq to populations across a wide altitudinal range and found genomic footprints of selection in association with certain climatic factors. In a second study, whole-genome resequencing was used to study the genomic basis of repeated altitudinal phenotypic divergence despite gene flow over microgeographic scales in continuously distributed populations [34]. The results indicate that the *A. halleri* system holds promise that has as yet been largely untapped.

Conclusion

Genomic studies of adaptive evolution performed recently in outcrossing *Arabidopsis* relatives, especially *A. arenosa*, show that the wild outcrossing species in this genus hold great promise for understanding a range of complex polygenic adaptations. Both transcriptomic and population genomic resequencing studies have yielded useful information, especially when combined. With a diversity of adaptations available in these allied outcrossing species, beyond what is observed in *A. thaliana*, they are poised to provide exciting new insights into adaptive evolution. And with the increase in genomic

resources in the *Arabidopsis* genus as a whole they are increasingly tractable model systems for understanding the genomic basis of adaptation.

Box 1: Why is selfing so problematic for studies of adaptation?

Arabidopsis thaliana is often used to study natural variation. However, *A. thaliana* is predominantly selfing, which not only presents serious technical difficulties for detecting targets of selection, but also has profound effects on the adaptive process itself: **Increased homozygosity** in selfers reduces genetic diversity and effective population size. This in turn hampers adaptation and causes drift to become an important factor in divergence among populations [35].

Reduced recombination in selfers increases linkage of adaptive sites to deleterious polymorphisms [36**,37]. The effect of linked variation is encapsulated in the Hill-Robertson effect, which posits that linked deleterious variants (even weak ones) can substantially dampen adaptation [38,39].

Background effects from unlinked genomic regions can similarly reduce adaptation rates, and this effect is far more potent in selfers (e.g. [40,41]). In an extreme case, an entire genome can hitchhike: recently Flood et al found fascinating evidence that selection on a chloroplast gene in *A. thaliana* facilitated the spread of a single lineage along the British railway system [42**]. This could not occur in an obligate outcrosser, where an absolute minimum of two parents must contribute to any novel lineage and thus genetic variants would continue to segregate in the population and can sort under selection. Both background effects and physical linkage to deleterious polymorphism create a "linkage drag" on selection because the units of selection become a homogenate across a wider portion of the genome in selfers and are thus less clearly positive or negative.

Fixation of maladaptive alleles increases on account of the conspiracy of increased linkage and drift [36**,37]. An elegant study in *A. thaliana* showed this clearly: using transplant experiments and QTL mapping to test the degree of local adaptation, genetic loci involved for an Italian and a Swedish *A. thaliana* accession and recombinants between them were investigated [43**]. The recombinant plants often outcompeted parents, especially in Sweden, and QTL mapping showed that some locally maladaptive alleles were fixed in the native populations.

Thus, while selfers do adapt, the combined effects of lower effective population sizes, increased homozygosity, reduced recombination, stronger drift, greater linkage of beneficial mutations to deleterious ones, and the higher rate of fixation of maladaptive alleles, presents serious biological obstacle to adaptation in addition to the more often discussed technical obstacles for interpreting patterns of differentiation in an adaptive framework. Furthermore, selfing species are more likely to fix recessive mutations, while outcrossers are more likely to fix dominant ones [44]. Since 85-90% of plant species are outcrossing [45], selfing is unrepresentative of the majority of plant diversity. Given these considerations, we strongly advocate that for studying mechanisms of adaptive evolution, and to allow studies to be more generalizable, outcrossing species are vastly superior models for understanding adaptive evolution.

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Figure 1: Habitat and morphological diversity in *A. arenosa*: This species is found in diverse habitats including (A) beaches, (B) high altitude mountain sites, (C) montane rock outcrops in forests, (D) ruderal sites including railways in central Europe. Plants from (E) a rock outcrop in the Rhine valley, (F) a rail platform in Germany, (G) a serpentine rock outcrop in the Alps, (H) a high altitude site in Romania, (I) a high altitude tundra site in the Alps, and (J) a lowland rock outcrop in the Pannonian basin of Hungary. Photos Filip Kolar, except C and F (KB).

Figure 2: Summary of demographic findings in A. arenosa: Summary of

demographic studies described in the text, showing both diploid and polyploid lineages (after whole genome duplication = WGD). Gene flow events are indicated by lines reaching from one lineage to another.

References.

- Novikova PY, Hohmann N, Nizhynska V, Tsuchimatsu T, Ali J, Muir G, Guggisberg A, Paape T, Schmid K, Fedorenko OM, et al.: Sequencing of the genus Arabidopsis identifies a complex history of nonbifurcating speciation and abundant trans-specific polymorphism. *Nat Genet* 2016, doi:10.1038/ng.3617.
- Schmickl R, Koch MA: Arabidopsis hybrid speciation processes. Proceedings of the National Academy of Sciences of the United States of America 2011, 108:14192–14197.
- Kolář F, Fuxová G, Záveská E, Nagano AJ, Hyklová L, Lučanová M, Kudoh H, Marhold K: Northern glacial refugia and altitudinal niche divergence shape genome-wide differentiation in the emerging plant model Arabidopsis arenosa. *Mol Ecol* 2016, 25:3929–3949.
- 4. Schmickl R, Paule J, Klein J, Marhold K, Koch MA: **The Evolutionary History of the Arabidopsis arenosa Complex: Diverse Tetraploids Mask the Western Carpathian Center of Species and Genetic Diversity**. *PLoS ONE* 2012, **7**:e42691.
- 5. Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML: Genome-wide genetic marker discovery and genotyping using next-

generation sequencing. Nature Genetics 2011, 12:499-510.

- 6. Arnold B, Corbett-Detig RB, Hartl D, Bomblies K: **RADseq underestimates** diversity and introduces genealogical biases due to nonrandom haplotype sampling. *Mol Ecol* 2013, **22**:3179–3190.
- 7. Arnold B, Kim ST, Bomblies K: Single Geographic Origin of a Widespread Autotetraploid Arabidopsis arenosa Lineage Followed by Interploidy Admixture. *Molecular Biology and Evolution* 2015, **32**:1382–1395.
- 8. Soltis DE, Soltis PS, Schemske DW, Hancock JF: **Autopolyploidy in angiosperms: have we grossly underestimated the number of species?** *Taxon* 2007, doi:10.2307/25065732.
- 9. Comai L: **The advantages and disadvantages of being polyploid**. *Nat Rev Genet* 2005, **6**:836–846.
- 10. Bomblies K, Madlung A: **Polyploidy in the Arabidopsis genus**. *Chromosome Res* 2014, **22**:117–134.
- 11. Bomblies K, Jones G, Franklin C, Zickler D, Kleckner N: **The challenge of** evolving stable polyploidy: could an increase in "crossover interference distance" play a central role? *Chromosoma* 2016, **125**:287–300.
- 12. Yant L, Hollister JD, Wright KM, Arnold BJ, Higgins JD, Franklin FCH, Bomblies K: **Meiotic Adaptation to Genome Duplication in** *Arabidopsis arenosa*. *Current Biology* 2013, **23**:2151–2156.

** A set of interacting genes that operate in prophase I of meiosis were found to show robust evidence of concerted selective sweeps in this genome scan of a young autopolyploid *A. arenosa*, when compared to diploid *A. arenosa*.

- 13. Hollister JD, Arnold BJ, Svedin E, Xue KS, Dilkes BP, Bomblies K: Genetic Adaptation Associated with Genome-Doubling in Autotetraploid *Arabidopsis arenosa*. *PLoS Genet* 2012, 8:e1003093.
- Storchova Z, Breneman A, Cande J, Dunn J, Burbank K, O'Toole E, Pellman D: Genome-wide genetic analysis of polyploidy in yeast. *Nature* 2006, 443:541– 547.
- 15. Yant L, Bomblies K: Genome management and mismanagement--cell-level opportunities and challenges of whole-genome duplication. *Genes & Development* 2015, **29**:2405–2419.
- 16. Wright KM, Arnold B, Xue K, urinova M, O'Connell J, Bomblies K: **Selection on meiosis genes in diploid and tetraploid** *Arabidopsis arenosa*. *Molecular Biology and Evolution* 2015, **32**:944–955.
- 17. Bomblies K, Higgins JD, Yant L: **Meiosis evolves: adaptation to external and internal environments**. *New Phytologist* 2015, doi:10.1111/nph.13499.

- 18. Baduel P, Arnold B, Weisman CM, Hunter B, Bomblies K: **Habitat-associated Life History and stress-tolerance variation in** *Arabidopsis arenosa*. *Plant Physiology* 2016, **171**:437–451.
- 19. Scholz H: Nomenklatorische und systematische Studien an Cardaminopsis arenosa (L.) Hayek. Willdenowia 2014, **149**:1–14.
- 20. Wang R, Farrona S, Vincent C, Joecker A, Schoof H, Turck F, Alonso-Blanco C, Coupland G, Albani MC: **PEP1 regulates perennial flowering in** *Arabis alpina*. *Nature* 2009, **459**:423–427.
- 21. Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF: **Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of Arabidopsis**. *The Plant Journal* 2004, **41**:195–211.
- Bieniawska Z, Espinoza C, Schlereth A, Sulpice R, Hincha DK, Hannah MA: Disruption of the Arabidopsis Circadian Clock Is Responsible for Extensive Variation in the Cold-Responsive Transcriptome. *Plant Physiology* 2008, 147:263–279.
- Merret R, Descombin J, Juan Y-T, Favory J-J, Carpentier M-C, Chaparro C, Charng Y-Y, Deragon J-M, Bousquet-Antonelli C: XRN4 and LARP1 Are Required for a Heat-Triggered mRNA Decay Pathway Involved in Plant Acclimation and Survival during Thermal Stress. *Cell Reports* 2013, 5:1279– 1293.
- 24. Przedpełska E, Wierzbicka M: Arabidopsis arenosa (Brassicaceae) from a lead-zinc waste heap in southern Poland a plant with high tolerance to heavy metals. *Plant Soil* 2007, **299**:43–53.
- Turisová I, Štrba T, Aschenbrenner Š, Andráš P: Arabidopsis arenosa (L.) Law.
 On Metalliferous and Non-metalliferous Sites in Central Slovakia. Bull Environ Contam Toxicol 2013, 91:469–474.
- 26. Eggler J: Ein Beitrag zur Serpentinvegetation in der Gulsen bei Kraubath in Obersteiermark. *Mitt. naturw. Ver. Steiermark* 1955, **85**:27–72.
- Arnold BJ, Lahner B, DaCosta JM, Weisman CM, Hollister JD, Salt DE, Bomblies K, Yant L: Borrowed alleles and convergence in serpentine adaptation. Proceedings of the National Academy of Sciences 2016, doi:10.1073/pnas.1600405113.

** This study integrated population genomics and ionomic elemental profiling to reveal candidates for both convergent evolution and adaptive gene flow mediating adaptation to multihazard serpentine soils in *A. arenosa.*

- Turner TL, Bourne EC, Wettberg Von EJ, Hu TT, Nuzhdin SV: Population resequencing reveals local adaptation of Arabidopsis lyrata to serpentine soils. *Nature Genetics* 2010, 42:260–263.
- 29. Hu TT, Pattyn P, Bakker EG, Cao J, Cheng J-F, Clark RM, Fahlgren N, Fawcett

JA, Grimwood J, Gundlach H, et al.: **The** *Arabidopsis lyrata* **genome sequence and the basis of rapid genome size change**. *Nature Genetics* 2011, **43**:476–481.

- 30. Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U: Evolution of metal hyperaccumulation required cisregulatory changes and triplication of HMA4. *Nature* 2008, **453**:391–395.
- 31. Meyer C-L, Vitralis R, Saumitou-Laprade P, Castric V: Genomic pattern of adaptive divergence in Arabidopsis halleri, a model species for tolerance to heavy metal. *Mol Ecol* 2009, **18**:2050–2062.
- 32. Roux C, Castric V, Pauwels M, Wright SI, Saumitou-Laprade P, Vekemans X: Does Speciation between Arabidopsis halleri and Arabidopsis lyrata Coincide with Major Changes in a Molecular Target of Adaptation? *PLoS ONE* 2011, 6:e26872–8.
- 33. Fischer MC, Rellstab C, Tedder A, Zoller S, Gugerli F, Shimizu KK, Holderegger R, Widmer A: **Population genomic footprints of selection and associations with climate in natural populations of** *Arabidopsis halleri* from the Alps. *Mol Ecol* 2013, **22**:5594–5607.
- Kubota S, Iwasaki T, Hanada K, Nagano AJ, Fujiyama A, Toyoda A, Sugano S, Suzuki Y, Hikosaka K, Ito M, et al.: A Genome Scan for Genes Underlying Microgeographic-Scale Local Adaptation in a Wild Arabidopsis Species. PLoS Genet 2015, 11:e1005361–26.
- 35. Charlesworth D, Wright SI: **Breeding systems and genome evolution**. *Current Opinion in Genetics & Development* 2001, **11**:685–690.
- 36. Hartfield M, Glémin S: **Hitchhiking of deleterious alleles and the cost of** adaptation in partially selfing species. *Genetics* 2014, **196**:281–293.

** A clear demonstration of the importance of considering linkage in selection models particularly in selfing species.

- 37. Hartfield M, Otto SP: **Recombination and hitchhiking of deleterious alleles**. *Evolution* 2011, **65**:2421–2434.
- 38. McVean GA, Charlesworth B: **The effects of Hill-Robertson interference between weakly selected mutations on patterns of molecular evolution and variation.** *Genetics* 2000, **155**:929–944.
- 39. Hill WG, Robertson A: **The effect of linkage on limits to artificial selection**. *Genetical Research* 1966, **8**:269–294.
- 40. Kamran-Disfani A, Agrawal AF: **Selfing, adaptation and background selection in finite populations**. *J Evolution Biol* 2014, **27**:1360–1371.
- 41. Arunkumar R, Ness RW, Wright SI, Barrett SCH: **The evolution of selfing is** accompanied by reduced efficacy of selection and purging of deleterious

mutations. Genetics 2015, 199:817-829.

42. Flood PJ, van Heerwaarden J, Becker F, de Snoo CB, Harbinson J, Aarts MGM: Whole-Genome Hitchhiking on an Organelle Mutation. *Current Biology* 2016, **26**:1–7.

** This study provided a striking description of the consequences of strong selection of a selfing species, *A. thaliana*. Here, the entire genome was dragged in linkage with a beneficial polymorphism, a phenomenon that could not occur in an obligate outcrosser.

43. Ågren J, Oakley JK, McKay JT, Lovell JT, Schemske DW: **Genetic mapping of** adaptation reveals fitness tradeoffs in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* 2015, **110**:21077-21082.

** A clear and well-reasoned study that coupled genetic mapping with transplant experiments in nature to show local adaptation as well as maladaptation in *A. thaliana*.

- 44. Ronfort J, Glémin S: **Mating system, Haldane's sieve, and the domestication process.** *Evolution* 2012, **67**:1518-1526.
- 45. Goodwillie C, Kalisz S, Eckert CG: **The Evolutionary Enigma of Mixed Mating Systems in Plants: Occurrence, Theoretical Explanations, and Empirical Evidence**. *Annu. Rev. Ecol. Evol. Syst.* 2005, **36**:47–79.





-Outcrossers are powerful models of adaptation with clear advantages over selfers.

-Arabidopsis arenosa is the subject of many of the most recent large-scale studies.

-Recent studies focused on adaptation to both environmental and intracellular challenge.