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Plant biology: Phylogenomics of mustards and their relatives

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A new nuclear phylogeny for the large family Brassicaceae opens the way for advanced comparative studies of adaptation, development, coevolution, hybridization, and diversification in this crucial group, which is the source for many of the genomic resources now used across the flowering plants.

The mustard family (Brassicaceae, ca. 4000 spp.) is of enormous scientific and economic interest. In 2000, *Arabidopsis thaliana* — a mustard with a small, 134-Mb genome[1] — was the first flowering plant to have almost its entire genome sequenced. Given that and its short generation time, *Arabidopsis* rapidly became the 'green *Drosophila*', a model organism that provided the foundation for our first detailed insights into the genetics of plant development, physiology, and interactions with animals, bacteria, and fungi[2]. Brassicaceae is remarkable for its large number of species, abundance in arid and disturbed environments, diversity in plant, leaf, and fruit form, high frequencies of whole-genome duplication, introgression, and hybridization, coevolution with butterflies that feed on them, and remarkable range of healthful crop plants[3–9]. The latter include broccoli, cauliflower, kohlrabi, cabbage, kale, and brussels sprouts — all domesticated from within just one species (*Brassica oleracea*) via selection for energy storage in different organs[10]. A similar range of domesticates has arisen within *Brassica rapa*, including turnips, bok choy, sarson, broccoli rabe, tatsoi, and mizuna[11]. All of these crops contain high concentrations of vitamins A, C, E, and K, as well as folate, calcium, iron, potassium, and phosphorus, combined with low caloric density and high levels of glucosinolates, a group of sulfur-bearing glucosides. In mustards, glucosinolates act as defenses against their herbivores[12] — and, indirectly, some competitors — and play a role in some species becoming invasive when introduced outside their range and those of herbivores, competitors, and competitors' mutualists adapted to their defenses[13,14]. But when consumed by humans, glucosinolates appear to protect against several cancers and chronic diseases affecting cardiac, neurological, and musculoskeletal systems[15].

The many species of Brassicaceae whose genomes have now been sequenced (91 currently in GenBank) make it a potential model family for addressing many issues, including the bases of morphological and physiological diversity, adaptation to environmental conditions, crop domestication, diversification of secondary chemistry, and roles of hybridization and whole genome duplication. Yet all these exciting applications require — to move from model species to part or all the family — a phylogeny, a detailed description of relationships among present-day species and their ancestors. Until now, a robust phylogeny including representatives of all 58 tribes of Brassicaceae recognized by German *et al.*[16] has proven elusive. Phylogenetic analyses based on morphology were misled by widespread convergence and hybridization[17], and early molecular phylogenies based on one or a few loci were often poorly supported (e.g., see Bailey *et al.*[18]). To derive a robust phylogeny for Brassicaceae — the birthplace of plant genomic resources — genomic data for hundreds of nuclear loci and dozens of plastid genes were needed.

Hendriks *et al.*, in this issue of *Current Biology*, provide the most comprehensive, strongly supported phylogeny to date for Brassicaceae, using model approaches for untangling relationships in large plant families, especially those involving extensive introgression, hybridization, or polyploidization[19]. Hybrid DNA enrichment, next generation sequencing, and maximum likelihood, coalescent, and network analyses were employed to infer relationships. Sequences of 1081 nuclear genes (including 353 enriched with the Angiosperm353 bait kit[20] now widely used across angiosperms, and 764 enriched with the Brassicaceae bait kit developed by Nikolov *et al.*[7]) and 60 plastid genes were obtained and analyzed for members of 319–322 genera of Brassicaceae, representing 57 or 58 of its 58 tribes. Remarkably, hundreds of nuclear genes were obtained from herbarium specimens, even some collected before 1900.

Hendriks *et al.* found support for four of the five supertribes recognized by Nikolov *et al.* and German *et al.*, but with evidence of extensive hybridization/introgression occurring within and among those supertribes. Some differences in deep relationships arose based on the extent to which nuclear loci showing evidence of paralogy (different copies of the same gene present, including different losses in different species) were excluded, and the nuclear and plastome trees showed extensive cytonuclear conflict. In all analyses, the monogeneric tribe Aethionemeae — herbs native to sunny limestone hillsides in Europe and western Asia (Figure 1) — is sister to the rest of the family. Supertribe Arabodae is resolved as two clades in the network analysis, and tribes Camelineae and Iberideae are polyphyletic in all nuclear trees. As is often seen in analyses involving hundreds of loci, bootstrap support values are 100% for many nodes that nevertheless show strong conflict among loci in the clades they resolve, as evidenced by low concordance

Figure 1. Phylogeny identifies a deep evolutionary split in the mustard family.
Aethionema saxatile, one of ∼60 species in the single genus forming supertribe Aethionemeae, sister to all other members of the mustard family Brassicaceae. (Photograph by Ghislain118/Wikimedia Commons (CC BY-SA 3.0).)

factors for those branches. Support for the backbone of the nuclear tree increases by culling loci that show evidence of paralogy, but the extensive cytonuclear conflict between the nuclear and plastome trees suggests that hybridization has been rampant (especially in 11 tribes identified by Hendriks *et al.*[19]) and that relationships in Brassicaceae may be better represented by a network. The superstrict nuclear tree — including 297 of 1081 loci that show the least evidence of paralogy — can be seen as an indication of the main non-reticulate relationships among species, but it is based on only 28% of the data.

Based on locus heterozygosity and allele divergence, Hendriks *et al.* argue that different loci and clades represent tree-like relationships, recent hybrids, old polyploids, high levels of polyploidy, and old and high levels of polyploidy. They found several 'rogue taxa' (mostly tribes) that jump into different places in the phylogeny based on different samplings of loci; two-thirds of the rogue samples seem to involve (allo)polyploidy, and another fifth have locus heterozygosity and allele divergence that border such a condition.

Finally, Hendriks *et al.* use the 20 genes with the most uniform rates of molecular evolution to estimate the stem age of Brassicaceae — the timing of its divergence from the ancestor of sister family Cleomaceae — as 38.8 million years ago, and its crown age as 24.5 Ma. This places the origin and early diversification of Brassicaceae during the greenhouse-to-icehouse transition, when tropical forests were largely replaced by temperate forests, scrubland, grassland, and deserts, all of which are common habitats for modern-day mustards and their relatives.

The new, time-calibrated Brassicaceae tree of life should be an important tool for comparative studies of genetic controls and evolution of development, morphology, chemistry, physiology, ecology, interactions with natural enemies, and rates of species and trait diversification, and for understanding the regulatory mechanisms underlying particular traits in crops, their wild relatives, and in feral forms that have escaped from cultivation and lost several of their human-selected traits.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Associative learning: Box jellyfish learns to avoid bumps

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Operant conditioning — learning to do something for a desired outcome — has never been convincingly demonstrated in Cnidaria. A study now shows that box jellyfish, *Tripedalia cystophora*, can learn to avoid bumping into an obstacle based on visual cues.

Jelllyfish, box jellyfish, hydra, corals, and sea anemones, making up the phylum Cnidaria, are often considered figuratively unbrainy because they literally do not possess the concentrated mass of neural matter that we would call a brain[1–3]. These animals are bedecked instead with nerve nets, a network of nerves spread throughout their body, with some conglomerations gaining the label of ganglia. Yet these animals learn, although most of the evidence to date has documented only non-associative learning.

Non-associative learning — habituation and sensitization — does not entail any association or mental connection between different types of stimulus. Instead, repeated presentation of a stimulus that elicits some behavior or some unrelated stimulus makes the animal respond less (in habituation) or respond more (in sensitization) to the behavior-eliciting stimulus[4]. A conglomeration of chemoreceptors and mechanoreceptors around the cnidocytes of Cnidaria are orchestrated to determine when to unleash an expensive nematocyst contained in the cnidocyte. The poison-tipped nematocyst, crucial for nabbing prey, is costly because it functions as a single-use gun: once fired, the entire cnidocyte needs to be replaced. A mechanical touch to the cnidocyte is not enough evidence of prey; it could instead be debris. But with some chemical evidence of a prey nearby beforehand, evidence for a prey nearby stacks up. The mechanisms of sensitization function as 'AND gates' in which a multiplicity of evidence for prey is needed before the disposable gun is fired.

Associative learning — classical conditioning and operant conditioning — entails linking different types of stimuli together, often thought to be a brainier kind of learning[5]. In classical conditioning, the learner links different events in the world, and in operant conditioning, the learner links its own behavior and its consequences. In Cnidaria, only two studies on classical conditioning, both in sea anemones, had adequate control conditions to rule out non-associative learning as an interpretation of the data[6,7]. Only hints of operant conditioning, in the form of avoidance learning in sea anemones, have been reported[8,9], one in 1905[8] and neither with convincing controls[2]. The sea anemones in these two studies might have avoided a proffered food that had led to nasty consequences in the past, either having a tweezer shoved down the throat[8] or getting