

High metabolic rates drive tiny genomes in plants (and birds): a commentary on ‘The smallest angiosperm genomes may be the price for effective traps of bladderworts’

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Flowering plants show a 2400-fold range in haploid nuclear genome size (Pellicer and Leitch, 2020). What is the functional significance of such extraordinary variation? Both cell cycle time and cell size increase with genome size, reflecting limits on DNA replication and metabolic control. Proximal causes of increases in plant genome size include polyploidy, spread of transposable elements and duplication within gene families; decreases result from rediploidization and losses of introns, intergenic spacers and transposable elements (Bennetzen and Wang, 2014; Soltis *et al.*, 2015; Wendel *et al.*, 2016), with some nuclear genes lost in parasitic and mycoheterotrophic species (Tmiltsena *et al.*, 2023). Ultimate causes of smaller plant genomes are thought to include: (1) short life cycles, which favour reduced DNA replication time (Carta *et al.*, 2022); (2) high mutation rates, which select for reduced mutation targets and repair costs (Gupta *et al.*, 2016; Bourguignon *et al.*, 2020); (3) high rates of recombination, which can purge transposable elements (Ren *et al.*, 2018); (4) N- and, especially, P-poor soils, favouring reduced allocation of these elements to nucleic acids (Šmarda *et al.*, 2013; Guignard *et al.*, 2016; Han *et al.*, 2021; but see Raven, 2021), which is potentially important given the high fraction of leaf P in nucleic acids (Suriyagoda *et al.*, 2023); and (5) selection for high photosynthetic rates, which require small cells to produce numerous small stomata, tightly packed mesophyll and high vein density and hydraulic conductance (Roddy *et al.*, 2020). Factors favouring larger plant genomes are less clear, but might include: (6) smaller effective population sizes, which increase drift and slow selective rediploidization and purging

of transposable elements (Lynch, 2011); (7) greater polyploidy at higher latitudes owing to increased formation of unreduced gametes at cooler temperatures (Mason and Pires, 2015); and, in my opinion, (8) greater incidence of vegetatively spreading herbs and shrubs, where frequent freezing works against seedling establishment. Such plants (not annuals or trees, in which vegetative spread is generally absent) can bypass meiotic failure during sexual reproduction in recent autopolyploids and permit their further evolution (Stebbins, 1971). Remarkably, nearly all the largest angiosperm genomes (1C > 30 Gb) occur in temperate herbs with massive storage organs, adapted to seasonal habitats and independently evolved in eight families of monocots (see data of Pellicer and Leitch, 2020). Geophytes also often have larger genomes than their non-geophyte sisters (Vesely *et al.*, 2013). I propose that large genomes might be favoured in geophytes to produce massive cells in which to store large starch granules with minimal investment in cell walls. The peak in angiosperm genome size at mid-latitudes reflects the large genomes of geophytes there (Bureš *et al.*, 2024: fig. 4).

None of these arguments, however, explains why 19 of the 20 smallest angiosperm genomes (65–140 Mb) occur in two sister genera of carnivorous plants with traps submersed in water or wet soils: *Utricularia* (bladderworts) and *Genlisea* (eel-trap plants) (Veleba *et al.*, 2014; Pellicer and Leitch, 2020). Substantially larger genomes (mean 1C = 596 ± 168 Mb) characterize third last genus of Lentibulariaceae: *Pinguicula* (butterworts), which bear sticky trap leaves above ground. Sequencing the *Utricularia gibba* genome revealed that, despite three sequential whole-genome duplications, it had contracted to 82 Mb via widespread deletion of gene copies, introns and transposable elements and by shortening of promoter regions (Ibarra-Laclette *et al.*, 2013). Carnivorous plants occur in nutrient-poor habitats (Givnish *et al.*, 2018), which should favour smaller genomes (see argument 4 above), but carnivores do not generally have smaller genomes than non-carnivorous relatives (Veleba *et al.*, 2020). The tiny genomes of bladderworts and eel traps thus pose a puzzle: are they simply a quirk of one small branch of the Tree of Life or are they the result of strong selection for reduced

genome size caused by their ecology and physiology?

Zedek *et al.* (2024) address this question, building on four key insights.

First, bladderworts respire at very high rates to pump water from their underwater traps and create a partial vacuum therein (Adamec, 2006). When a small aquatic animal (e.g. *Daphnia*) bumps into the trap door at the mouth of the bladder, it pushes the door over a confining threshold; overpressure outside the trap then pushes water and prey inside in a few milliseconds, followed immediately by the door snapping shut and, later, secretion of digestive enzymes and digest resorption (Plachno and Muravnik, 2018). High rates of energy supply are needed to set these bladder-traps.

Second, cytochrome *c* oxidase (COX), a mitochondrial enzyme that plays a crucial role in generating energy in aerobic organisms, has undergone positive selection for a double cysteine (CC) motif in COX I subunit, helix 3 of these plants (Jobson *et al.*, 2004). This substitution occurs only in *Utricularia* and *Genlisea* (and, oddly, the desert gymnosperm *Welwitschia*) in >33 000 COX sequences examined.

Third, this CC substitution can substantially increase the power delivered by COX by decoupling electron transport and proton pumping (Laakkonen *et al.*, 2008).

Finally, Albert *et al.* (2010) argued that proton sequestration in the mitochondria is imperfect and that leakage can form reactive oxygen species (ROS). ROS can damage DNA at the nucleotide and whole-helix levels, including double-strand breaks leading to deletions or insertions. Higher mutation rates should favour smaller genomes (see argument 2 above), hence selection for greater power to drive bladder-traps should lead to the CC mutation, greater metabolic rates, increases in mutation rates and smaller genomes. Albert *et al.* (2010) argued that selection favoured greater mitochondrial power in *Genlisea* too, despite it having open underwater traps with spiral arms leading prey to a digestive chamber through nested cones of stiff hairs that function like an eel trap or lobster pot, perhaps to pump water out of the chamber to suck more water slowly in, in order to attract protozoan prey (Barthlott *et al.*, 1998) and prevent digest from diffusing

into the surrounding water (Meyers-Rice, 1994). In my view, selection for such pumping in *Genlisea* and the open-chambered ancestor of *Genlisea*–*Utricularia* might have been even stronger than in *Utricularia* itself.

Zedek *et al.* (2024) test the hypothesis of Albert *et al.* (2010) by sequencing COX1 sequences and compiling genome and chromosome sizes for dozens of species of Lentibulariaceae. They found that, as predicted, species with the ancestral COX1 sequence [with a lysine-serine (LS) motif at positions 113 and 114] had larger genomes and chromosomes than those with either the CC or CS motif. The CS motif characterizes *Genlisea* sect. *Genlisea*; CC, *Utricularia* and paraphyletic *Genlisea* section *Recurvatae*; and LS, *Genlisea* section *Tayloria*, *Pinguicula* and outgroups. Taking phylogeny into account, Zedek *et al.* showed that species with the CC or CS mutation had significantly smaller genomes and chromosomes than those with LS. Species in *Genlisea* section *Genlisea* with the CS mutation had significantly smaller chromosomes ($P < 0.044$) than species with the CC mutation, but the smaller genomes of CS species did not differ significantly ($P > 0.144$) from those of CC species. Zedek *et al.* found no significant difference in target genome size among groups with the CC versus CS mutation, but this might reflect sample size: only eight species in *Genlisea* section *Genlisea* carry the CS mutation. But six have smaller genomes than almost all other plants, consistent with the CS mutation in open-trapped *Genlisea* yielding even greater power, ROS production and selection for smaller genomes than CC. The trade-off between COX power and damage caused by ROS could have led COX1 motif evolution from LS in *Pinguicula* and all outgroups via a single mutation to CS in the open-trapped ancestor of *Genlisea*–*Utricularia*, to maximize water pumping, then to CC in *Utricularia* and *Genlisea* section *Recurvatae*, to allow pumping while reducing ROS damage.

These findings call for new studies to see whether CC and CS motifs increase maximum energy delivery, water pumping, ROS and double-strand DNA breaks, repairs and recombination. Editing the COX1 mitochondrial sequence in all three genera of Lentibulariaceae would provide an elegant experiment to connect the CC, CS and LS motifs directly to those processes. For now, CRISPR cannot be used to do this, given the inability to move guide RNA through the mitochondrial double membrane (Gammage *et al.*, 2018). But alternative approaches, including base editors and transcription-activator-like

effector deaminases, might soon provide efficient means for editing mitochondrial DNA (Lim, 2024). Even without such editing, it would be useful to explore the connection between possessing CC, CS or LS and whole-plant energy balance (photosynthesis–respiration). Increased respiration in CC plants must be less than the elevation of photosynthesis if CC is to be favoured; this trade-off could also shape COX1 evolution. We also need to see whether water is pumped out of the digestive chamber in *Genlisea*, whether it prevents most digest from escaping and whether pumping is greater in *Genlisea* section *Genlisea* than in section *Recurvata* and greater in section *Recurvata* than in section *Tayloria*, as expected from their genome sizes. Such measurements are essential to determine whether the hypothesis of Albert *et al.* (2010), developed for *Utricularia*, also applies to *Genlisea*.

Nucleotide substitution rates in the nuclear, plastid and mitochondrial genomes in *Genlisea* and *Utricularia* are greater than those in larger-genomed *Pinguicula* (Jobson and Albert, 2002), consistent with the metabolic rates–ROS hypothesis. Variants of this hypothesis might help to explain genome evolution in other groups. For example, bird genomes are substantially smaller than those of other amniotes; this has been viewed as resulting from selection for the higher metabolic rates needed for powered flight, favouring smaller cell sizes and thus smaller genomes (Hughes and Hughes, 1995; Watari and Edwards, 2002). A metabolic rates–ROS hypothesis might provide another explanation, with selection for higher metabolic rates causing increased ROS production and DNA damage, selecting for smaller genomes. Either argument could explain why avian genome size decreases with metabolic intensity, falling with the relative sizes of the heart and flight muscles and increasing with body mass and wing loading (Wright *et al.*, 2014). The smallest genomes occur in hummingbirds, the largest in ostriches. The supposed dependence of larger genomes and accumulations of transposable elements in flightless birds (which are often large and might thus have small populations) on drift (Bravo *et al.*, 2021) might equally be ascribed to a trade-off between metabolic power and ROS damage. Careful measurements of ROS and mutation rates in exons in birds as a function of body size, metabolic intensity and flying ability, together with effective population sizes in flying and flightless relatives (e.g. in tinamous vs. rheas or emus), would help to distinguish between these hypotheses.

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