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# There's much left to learn
# *Clethra's* Chromosomes

By W. John Hayden, Botany Chair

Many would argue that chromosomes, genes, and DNA form the ineluctable essence of modern biology. Not only do these fundamental components of living cells provide moment-to-moment instructions by which cells carry out basic life processes, they also control inheritance of characteristics from one generation to the next. These essential functions of DNA stem from its repetitive structure. Hugely long DNA molecules are built from just four components, referenced by their single-letter abbreviations, A, C, G, and T. It is the specific sequence of these As, Cs, Gs, and Ts that constitutes the coded information of DNA. Moreover, molecular biologists have determined that this genetic code is universal, i.e., petunias, earthworms, chimpanzees, and everything else spell out their genetic instructions using the same three-letters-at-a-time code words. We are now well into the genome era, in which biologists seek meaning by reading, for many organisms, the codes contained in the long strings of As, Cs, Gs, and Ts that encompass the totality of their DNA.

Chromosomes were of interest to biologists, however, long before anyone ever dreamed of DNA sequencing or reading entire genomes. Chromosomes have been an object of intense scrutiny since the details of cell division—mitosis and meiosis—were elucidated in the late 19th century. An early insight connecting chromosomes with the mechanism of heredity derived from the observation that gametes (sperm, eggs) of plants and animals contain half the number of chromosomes present in other, nonreproductive cells of the same species. Although the actual number of chromosomes present varies from species to species, the general terms haploid and diploid refer, respectively, to this distinction between reproductive and vegetative cells; haploid gametes have only a single set of chromosomes, whereas diploid cells have two.

An early publication (Winge 1917) established that chromosome numbers in closely related plants often follow predictable patterns. For example, the chromosome number in species of the genus *Chrysanthemum* has been shown to vary widely; some species have 18 chromosomes per cell, others 36, still others 54, or 72, or 90. (These are all nongamete-related counts from diploid cells.) The realization that counts from these related species were all multiples of the number nine led to the concept of polyploidy, the occurrence of multiple full sets of chromosomes in series of related species.

Chromosome counts are made from cells caught in the act of cell division. A commonly used source is the actively growing root tip, with cells caught in late prophase or metaphase of mitosis (ordinary cell division). Root cells have two sets of chromosomes, one full set received from each parent; a count made from a root tip thus yields what is called a diploid (2n) count. Alternatively, chromosomes can also be counted from anther cells undergoing meiosis before pollen formation. (Meiosis reduces the chromosome number by half, a process that precedes gamete formation. In most plants, including *Clethra*, sperm cells do not form until after pollination, when the pollen grain has formed a pollen tube inside the style.) If observed at the right stage (late prophase I or metaphase I of meiosis), all the chromosomes are tightly paired, so close together, in fact, that each pair appears through the microscope as a single body. A count made at this stage is called a haploid (n) count because the number of chromosomes observed will match the reduced number that will be found in the gametes that soon form.

Available information indicates that the genus *Clethra*, in which Sweet Pepperbush, the 2015 VNPS Wildflower of the Year, is classified, contains a polyploid series (see summary box). So far, four species, *C. alexandri*, *C. arborea*, *C. lanata*, and *C. pringlei*, are reported to have a diploid chromosome number of 16. Virginia's two species, *C. acuminata* and *C. alnifolia*, have a diploid count of 32. And *Clethra barbinervis*, from Japan and sometimes cultivated in botanical gardens, is reported to have a diploid count of 80.

Clearly, for the genus *Clethra*, the base chromosome number (often designated as x) is eight, and each species in the series has some multiple of eight for its total chromosome count in vegetative cells.

At this point the terminology gets a little tricky, because the word diploid can be used in two contexts, with different meanings. As mentioned above, a diploid cell, in a life-cycle frame of reference, denotes a nonreproductive cell containing twice as many chromosomes as found in pollen and eggs. But the concept of diploid can be extended to apply to species at the base of a polyploidy series.

A diploid species refers to a species that has just two sets of chromosomes in vegetative cells; similarly, a triploid species would have three sets, and a tetraploid species would have four, etc. *Clethra arborea*, for example, is considered to be a diploid species because cells of its roots, stems, and leaves contain 16 chromosomes (two sets of eight). (These would also be diploid cells in the life-cycle context, and its eggs and sperm would be haploid, with one set, eight chromosomes, each.) Moving up the polyploid series, *C. alnifolia* is a tetraploid species; its nonreproductive cells (diploid in the life-cycle context) contain 32 chromosomes (four multiples of the base chromosome set of eight), while sperm and eggs have 16 chromosomes. *Clethra acuminata* is also considered a tetraploid species. But *C. barbinervis* is a decaploid species, with 10 sets of chromosomes (= 80) in nonreproductive cells and 5 sets of chromosomes (= 40) in gametes.

Generally speaking, polyploid series start at the diploid level, and, by various mechanisms, higher chromosome counts arise over time. Almost certainly, then, ancestral *Clethra*, like modern *C. arborea*, was diploid, 2n = 2x = 16. It should also be certain that the ancestry of *C. alnifolia* and *C. acuminata* includes at least one jump, from the diploid to the tetraploid level. And who knows what complex path may have occurred in the ancestry of decaploid *C. barbinervis*. But whatever the details, the trend was from fewer to more whole sets of chromosomes.

Little more can be inferred from the data at hand because, of the 65 or so known species of *Clethra*, chromosome counts have been published only for the seven mentioned in the summary box. In other words, nearly 90 percent of the species of *Clethra* are unknown in terms of this fundamental aspect of their biology. We don't know how many of the 58 species without published chromosome counts will prove to be diploid species. We don't know if tetraploid species are common or whether *C. alnifolia* and *C. acuminata* are the only two. And we don't know if there are any species of *Clethra* alive today that bridge the gap between tetraploids (2n = 4x = 32) and the decaploid (2n = 10x = 80) status of *C. barbinervis*. Nor do we know whether any living *Clethra* species has a chromosome count greater than 80. Clearly, we know only a little, and we don't know a lot. But the little that we do know about chromosome numbers in *Clethra* hints at a rich story waiting to be uncovered. ❖

## Known Chromosome Counts in *Clethra*: A Summary

DIPLOID (2x) SPECIES, n = 8, 2n = 16
*C. arborea,* from Madeira (Hagerup 1928)
*C. alexandri,* from Jamaica (Judd 2008)
*C. lanata,* from Mexico (Kyhos 1965)
*C. pringlei,* from Mexico (Reed 2005)

TETRAPLOID (4x) SPECIES, n = 16, 2n = 32
*C. acuminata,* from eastern North America (Tanaka & Oginuma 1980)
*C. alnifolia,* from eastern North America (Tanaka & Oginuma 1980)

DECAPLOID (10x) SPECIES, n = 40, 2n = 80
*C. barbinervis,* from Japan (Tanaka & Oginuma 1980)

Illustration of *Clethra alnifolia* by Lara Call Gastinger from *Flora of Virginia*. © Flora of Virginia Project

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