

13andMe

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Over 5 million people have had their DNA analyzed by the genetic services company 23andMe. This analysis can provide information about someone's ancestry by examining how closely their DNA matches the DNA of people from different geographic regions. The genetic profile can predict, with some accuracy, the likelihood for certain traits like eye color or aversion to cilantro. The presence of DNA from closely related species, such as *Homo neanderthalensis*, can also be determined.

The techniques used to explore the genomes of humans can be applied to azaleas to understand their genetic diversity and to identify genetic traits of horticultural interest. In humans, unique DNA sequences or "markers" have been identified that are distinctive of a geographic region or physical trait. DNA markers are often single nucleotide changes, such as an A (adenine) in place of a G (guanine). These variants are called SNPs, for "single nucleotide polymorphisms". Through genome sequencing, SNP markers have been identified across the 23 pairs of human chromosomes. We were interested in looking at the genetic diversity of Piedmont azalea (*Rhododendron canescens*), which has 13 pairs of chromosomes.

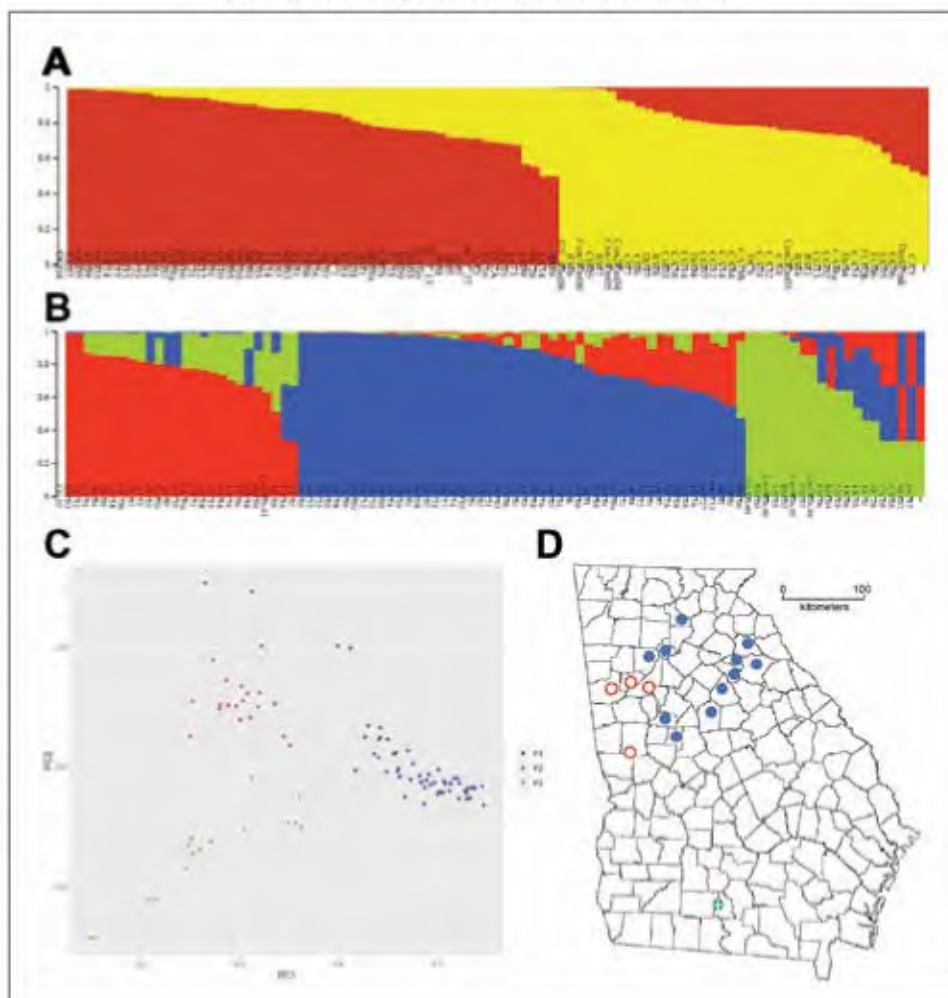
With support from the Azalea Research Foundation, we collected leaves from about 300 Piedmont azalea plants from across Georgia and isolated their DNA.¹ Ninety-six plants were selected to represent the diverse collection sites, and their DNA was cut into specific fragments by two enzymes. The ends of the DNA fragments were sequenced, providing a sampling of each genome that could be compared between the 96 plants. Around 100 million high-quality sequences were read, and SNPs were identified using two different bioinformatic platforms, STACKS² and GBS-SNP-CROP³. Over 3,000

SNP markers were then used to determine how closely related the 96 Piedmont azaleas were to one another.⁴

The relatedness of plants in the azalea collection is shown in Figure 1. Plants with similar patterns of SNP variation were assigned to groups, shown here as colored vertical bars. Several azalea plants had genetic characteristics of more than one group. The number of groups identified with matching SNP patterns depended on which bioinformatic program was used to identify SNPs. Two major groups (red and yellow) were resolved from the STACKS data (Fig. 1A). Three groups (red, blue, green) were found with the GBS-SNP-CROP data (Fig. 1B).

The patterns of SNP variation were then examined by a different approach called principle component analysis (PCA). PCA compared the azalea samples based on the two major sources of that variation, identified as principal component 1 and 2 (PC1 and PC2) in Fig. 1C. In this case, SNP markers from both bioinformatic programs gave the same answer: three populations could be distinguished, corresponding to samples from (a) the western Georgia Piedmont, (b) the eastern/central Georgia piedmont, and (c) collaborators (Fig. 1D). The samples from collaborators came from locations in northern Florida, southern

▼ Figure 1—Population structure of Piedmont azalea using data from STACKS analysis (A) or GBS-SNP-CROP analysis (B). Principle component analysis (C). Collection sites of Piedmont azaleas (D). The groups identified by the blue, red, and green colors in figures B, C, and D are the same.



Georgia, and northern Georgia. This population, interestingly, contained all plants with a particular phenotypic variation, the presence of glandular hairs on their petioles, bud scales, and new growth.

A third type of population statistics⁵ determined that the genetic diversity of Piedmont azaleas within each of the three populations was high. In fact, more variation was found among the individuals than between the populations. This suggests that there may be a significant level of gene flow between the populations. The degree of inbreeding among the Piedmont azaleas was found to be low, which would be expected from a species in which crossing among unrelated individuals is favored. Chappell and co-authors⁶ proposed that pollinators may be responsible for reducing differences between populations of native azaleas by increasing gene flow. Piedmont azalea has been observed to be pollinated by bees, butterflies, and hummingbirds. Hybridization with other native azaleas species, such as *R. flammeum*, may be a source of genetic diversity in *R. canescens*.⁶

The level of genetic diversity of Piedmont azalea in Georgia is encouraging for the search for variation in genes for traits of ornamental potential. We are interested in genes that control plant shape, particularly height and branching patterns. We are in the process of screening over 200 of the Piedmont azaleas for variation in 15 genes that are known to regulate height and branching in other plant species. These include genes involved in hormone biosynthesis and signaling, lateral bud repression, and the transition from vegetative growth to flowering. A dwarf Piedmont azalea is among the plants being screened and this analysis may discover the genetic basis for its reduced stature. Plants with variation in specific genes may be useful for breeding Piedmont azaleas with a more compact plant shape for landscaping.

References

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Dayton Wilde is a professor and Lav Yadav is a doctoral student in the Horticulture Department of the University of Georgia. A major interest of our lab is the application of genetics to streamline the development of ornamental traits, particularly in native plants. The authors wish to thank the Azalea Research Foundation for its generous support of this research.

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