

Stalking Wild Azalea Mutants in Georgia and Alabama

By Dayton Wilde and Jim Gegogine—Athens, Georgia

The diversity we see in azaleas results from variation in genes and genomes. Mutation, hybridization, and multiplication of azalea genomes have produced variation in gene sequences, gene combinations, and gene regulation. In the wild, such as it is, there is selection for genetic variation that increases the viability of a plant and its progeny. Genetic mutations that cause changes that are disadvantageous in nature can be maintained if they are compensated by a good copy of the gene. It is these recessive genes that interest us because of their potential to develop ornamental traits in azaleas that would rarely be found in the wild.

Recessive genes have been used to develop ornamental traits for centuries, although not deliberately. Repetitive flowering in modern roses is due to a knockout mutation that was present in Chinese cultivars around 1000 AD and bred into European roses 800 years later.¹ Double-flowered morning glories cultivated in 18th century Japan were the result of a mutation in a gene, *agamous*, that regulates flower development.² When neither copy of *agamous* is functional, stamens are converted to petals. *Agamous* mutations have also been found to be responsible for double-flowers in modern cultivars of ornamental cherry, gentian, and rue anemone.³⁻⁵ Due to the loss of functional stamens, double-flowering is an example of a trait of ornamental interest that would seldom be observed in the wild. A recessive *agamous* mutation could be present in plants that appear normal, though. We are interested in looking for variant copies of genes leading to architectural traits (dwarfing, for example) that would be rarely seen in azaleas growing naturally.

To search for natural mutations in native azaleas, a genetically diverse population of plants is needed. An advantage of working with native species is that, by definition, we are at the center of their diversity. *Rhododendron canescens*, or Piedmont azalea, seemed like a reasonable species to start with. It is the most common native azalea in the South, which makes finding a large number of plants easier. Piedmont azalea was found to have a high level of genetic variation, due in part to introgression from other native azalea species.⁶ It is the first native azalea to bloom in many areas and is important to native pollinators in early spring. It is hardy, exhibits lace bug resistance,⁷ and is used in the horticultural trade. We wanted to screen Piedmont azaleas for natural mutations in key genes of plant architecture, particularly height and branching.

How many Piedmont azalea plants need to be screened to identify functional mutations? This depends on the genetic diversity of the collection, the number of genes being examined, and how lucky you are. We have screened for natural mutations in blueberry, a relative of azalea in the *Ericaceae*. We were looking for variation in *TFL1*, the gene whose mutation causes repetitive flowering in roses

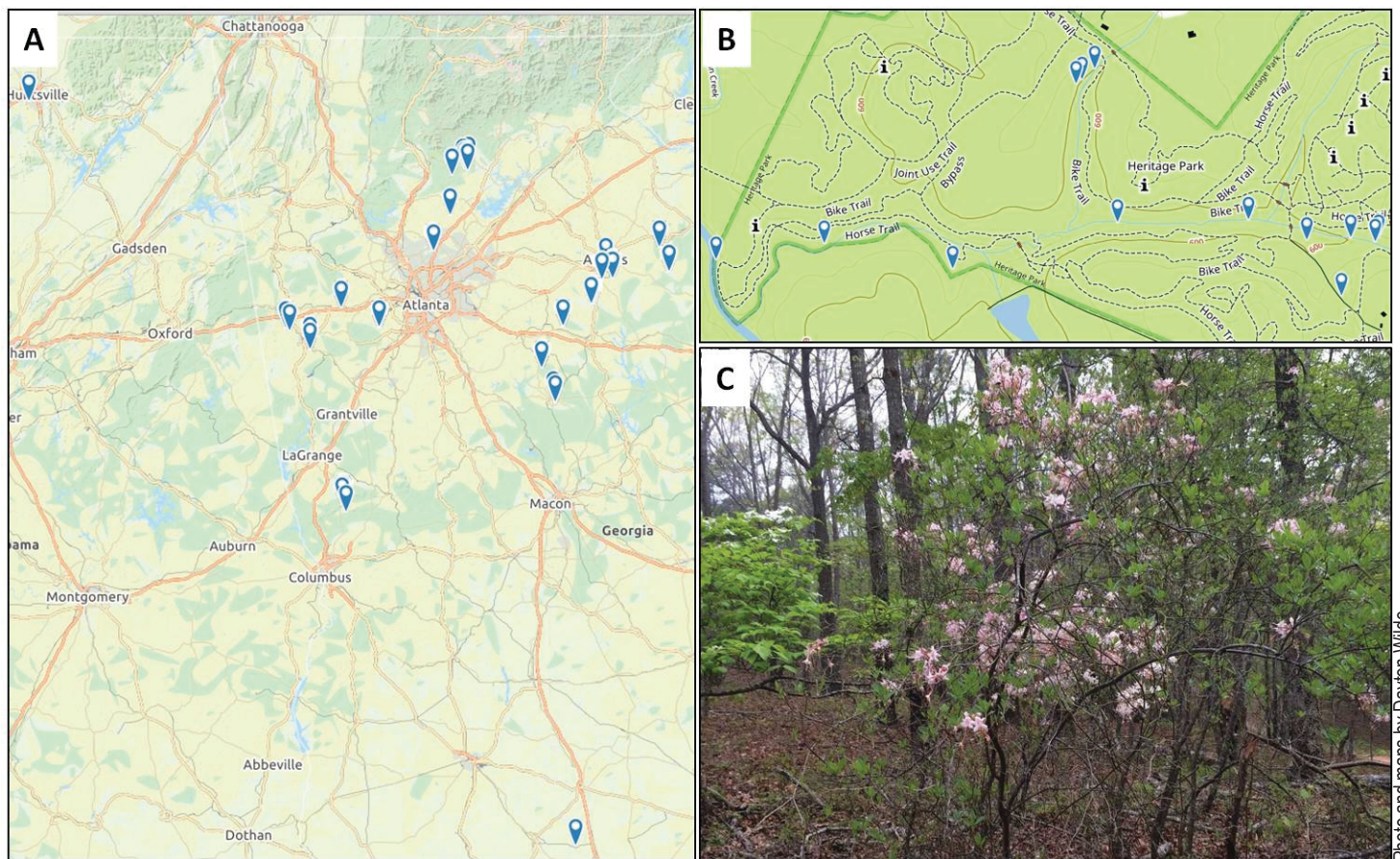
(and other plants). We examined 160 blueberry lines from the USDA germplasm collection and detected one line with a defective *TFL1* allele.⁸ Screens of germplasm collections have identified gene mutations for agronomic traits in rapeseed (117 lines), corn (175 lines), chickpea (192 lines), barley (210 lines), sugar beet (268 lines), and rice (392 lines), among others.⁹ For Piedmont azalea, our goal was to obtain material from 200-300 plants from diverse locations.

With the help of botanical societies, state and county agencies, and numerous individuals, we obtained leaf samples from 290 Piedmont azaleas in the spring of 2016. Most of these young leaves were collected across Georgia and kept cool until frozen and stored at -80o Celsius. Some Piedmont azalea samples were collected by collaborators and preserved by desiccation with silica gel.¹⁰ The GPS location, flowering date, altitude, and photographs were recorded for most plants using a cell phone with a Gaia GPS mapping app.¹¹ The origin of a few azaleas that had been transplanted is unknown.

A map of the sample locations was developed using Gaia GPS tools. Figure A shows the location of the collection sites in Georgia and Alabama. Within each site, the location of a sampled plant is marked on the map with a pin (Fig. B). Leaves from up to 15 plants were collected per sampling site. Most plants were located in moist areas along creeks, although some were found in drier, upland environments (Fig. C). If genetic analysis identifies an azalea with a mutation, GPS coordinates and photographs should allow us to return to that plant.

Once all of the leaf samples were collected, DNA was isolated. Frozen leaves were ground in a bead mill, in which plastic tubes containing metal beads and the samples were shaken at very high speed (30 oscillations/second). DNA was purified from the powdered tissue with a commercial kit (DNeasy) that had been used to isolate DNA from silica-dried leaves of *Rhododendron aganniphum* and *R. phaeochrysum*.¹⁰ The purity and concentration of the DNA was determined and the samples were stored at -20o C.

The development of the Piedmont azalea collection was funded by the ASA Azalea Research Foundation Committee and this collection was a major factor in acquiring further support to examine variation in genes controlling architectural traits. Through a Specialty Crop Block Grant from Georgia Department of Agriculture, we will screen the collection for mutations in key genes controlling height and branching patterns. Samples of Piedmont azalea DNA will be examined by the same techniques used to screen for disease-causing mutations in human genes.¹² The plant genes of interest are ones whose mutations have been important in the domestication of several crop species. We will look at genes that are normally responsible for making the hormone



▲ Piedmont azalea collection. (A) Collection sites in Georgia and Alabama. (B) Location of plants within a collection site. (C) Photograph linked to GPS coordinates of a sampled plant.

gibberellin, controlling axillary bud growth, and repressing the initiation of flowering. Mutations in these genes have led to semi-dwarfism, increased branching, and continuous flowering, respectively. We will determine the frequency of variation in these domestication genes. If Piedmont azaleas are found with mutations affecting gene function, the plants will be brought into an azalea breeding program at the University of Georgia.

The Piedmont azalea collection could also be used to better understand *R. canescens* as a species. Previous research identified AFLP markers that could detect genetic variation within and among seven species of the *Rhododendron* section *Pentanthera*, including *R. canescens*.⁶ These markers can be used to examine the genetic composition of the *R. canescens* population and how it is influenced by introgression from other species. Markers may also be used to investigate genetic differences related to morphological variation found within *R. canescens*, such as the presence of glandular hairs. Genetic variation in this azalea collection could be of both horticultural and scientific value.

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Dayton Wilde is a professor and Jim Gegogine is a research technician 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.

Three Exquisite Satsukis



Photo Jim Trumbly

▲ 'Gyoko'



Photo Jim Trumbly

▲ 'Tomenishiki'

▼ 'xMiyoshino'



Photo Jim Trumbly



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