

Getting more mileage from microsatellites

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They say you only find what you're looking for, and that applies in population genetics as well as in life. Population genetic studies rely on scoring known, characterized variation in DNA in order to decipher the history of different populations. However, this known variation may not be sufficient to give a properly resolved picture in every species. In research presented in a recent issue of *Applications in Plant Sciences*, Dr. Juan Viruel and colleagues used next-generation sequencing (NGS) to identify additional variation in DNA markers of the carob tree, *Ceratonia siliqua*. This study shows that increasingly affordable NGS technologies can reveal the history of this important tree in higher resolution than possible with traditional methods.

"The carob tree has been neglected by [population genetic studies](#) despite its historical and economical importance in Mediterranean societies," said Dr. Viruel, Research Leader in Conservation Genetics at the Royal Botanic Gardens, Kew, and the lead author of this study. Population genetic studies, which analyze the genetic variation across multiple populations of the same species, can reveal patterns of migration, isolation, divergence, and interbreeding across space and time, as well as other questions about the history of a species. This particular study on the population genetic structure of the carob tree is part of a larger project that, according to Dr. Viruel, "investigated the [genetic diversity](#) and structure of the carob trees and their root symbiont community to characterize the specificity of symbiotic interactions at the Mediterranean scale."

However, species differ in the amount of genetic variation present in

their genomes, as well as how well characterized that variation is. In particular, "bottlenecks," or periods of very small population size, reduce [genetic variability](#) and make it harder to get sufficient resolution in population genetic studies. "To investigate the genetic diversity of a tree species that underwent a strong bottleneck in the past, we needed to focus on genomic regions having the fastest evolution rate, i.e., microsatellites," said Dr. Viruel.

Microsatellite markers, also known as simple sequence repeats, are often analyzed because of their fast rate of evolution, and consequent high degree of variability in populations, which helps in parsing the distribution of genetic variation. Typically, the scoring method relies on the size of the amplified region, or "amplicon," a proxy for the number of repeats present. However, in cases of species that underwent a strong bottleneck, the repeat number in microsatellites might be insufficient to resolve population genetic questions.

In such cases, the authors show that using an NGS approach to discover additional variation in microsatellites can sharpen the resolution of how DNA varies across populations. "In our study, we found additional sources of polymorphism (specifically, single-nucleotide polymorphism and insertion/deletion polymorphisms) in 87% of the loci analyzed," said Dr. Viruel. "Studies dealing with very limited genetic diversity...could be benefited by incorporating sequence information."

The cost of the NGS technologies used to discover this additional variation is dropping rapidly, making it much more feasible to conduct this type of research. "The costs of our NGS-based method to detect polymorphisms in [microsatellite] amplicons is comparable to the costs of using the traditional size scoring when working with a big number of samples," said Dr. Viruel.

"I'm excited about the questions that we can answer with this approach,"

said Dr. Viruel. "We have significantly increased the variation detected in carob populations throughout the Mediterranean." The increased resolution afforded by that additional variation is already paying off. "We have found very interesting phylogeographic patterns that will refute previous hypotheses about its origin in the Mediterranean," said Dr. Viruel. "But we will tell that story in the next paper."

More information: Juan Viruel et al, Advances in genotyping microsatellite markers through sequencing and consequences of scoring methods for *Ceratonia siliqua* (Leguminosae), *Applications in Plant Sciences* (2018). [DOI: 10.1002/aps3.1201](https://doi.org/10.1002/aps3.1201)

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