
20th Annual James J. Whalen Academic Symposium
Population Genetics of *Scaevola* in the islands of Puerto Rico

Adriana Morales and Susan Witherup (faculty adviser)
Department of Biology
Ithaca College, Ithaca, NY

A number of studies have introduced the idea that island species have low levels of genetic diversity, but current research suggests that levels of genetic diversity cannot be predicted due to the different environmental conditions on each island [1]. *Scaevola plumieri* and *Scaevola taccada* grow in coastal shrub lands in the Islands of Puerto Rico (PR). They can be found along rocky, sandy and coralline shorelines [2]. *S. plumieri* is locally common and native to the Islands of PR. *S. taccada* was introduced to the Caribbean in the 1970s and was first recorded in Vieques, a Caribbean island off Puerto Rico's eastern coast, in 2002 [3]. Since its arrival to the Caribbean *S. taccada* has been observed to outcompete other species in its habitat including *S. plumieri* [4]. *S. plumieri* has not only been threatened by *S. plumieri* but also by natural events, such as hurricanes or extreme drought, but also by habitat loss from human activity. Vieques has been dramatically impacted by the removal of native flora in the beginning in the nineteenth-century, and later by extensive deforestation occurred to provide space for sugarcane plantations. From 1943 to 2003, the U.S Navy used the island as a Naval training range [5]. These activities ultimately may also have contributed to a loss of genetic diversity in *S. plumieri*, via habitat fragmentation. Pollination data from three consecutive years suggests that the introduced species, *S. taccada*, utilizes similar pollinators than the native species, but *S. plumieri* ultimately had higher visitation rates and attracts several native pollinators.

My research focuses on documenting the occurrence of *Scaevola* populations in the islands of PR and exploring the possible impact of an invasive congener, *S. taccada*, on the number, range, and genetic diversity of the native populations. Microsatellite markers will be used to estimate the levels of genetic diversity within *S. plumieri* and *S. taccada*. The goals of the study are to (1) evaluate genetic diversity of intra- and inter- populations of *Scaevola* in the Puerto Rican islands, (2) assess gene flow within and among populations of *Scaevola*, (3) determine if Vieques has a greater genetic diversity than the main island of Puerto Rico, and lastly (4) test if genetic variance correlates with geographic distances among the Puerto Rican Islands.

Leaf samples for both species were collected from 12 different locations in the islands of PR (**Figure 1**) and genomic DNA was extracted using the Gene Jet Plant DNA Purification Kit (Thermo Fisher Scientific). The levels of genetic diversity of *S. plumieri* and *S. taccada* plant species were measured using microsatellite markers. Microsatellites, also called short tandem repeats (STRs) are short repetitive sequences that are susceptible to rapid mutations. These polymorphic DNA sequences contain two or three nucleotide repeat sequences, and the number of repeat units in a locus may be different resulting in alleles of numerous lengths [2,6]. By using polymerase chain reaction (PCR) we can amplify the marker of interest. Thirty-nine primers pairs developed for *S. taccada* [7,8,9] were tested for utility in *S. plumieri*. One forward primer was modified (Stac 6M). Four of these primers produced a PCR product, but only two revealed polymorphisms in repeat length among individuals. Using the polymorphic regions, we used Arlequin 3.5, an integrated software for population genetic data analysis [10], to test for different parameters, such as analysis of molecular variance (AMOVA). AMOVA tests the variance among groups, among populations within groups and within populations, and it also calculates an equivalent of F_{st} between groups. We also tested for Hardy-Weinberg equilibrium (HWE) by comparing the observed and expected heterozygosity between the two population and their different locations. Lastly, a Mantel test was

performed to determine if the samples had a similar genetic variance between the islands of Puerto Rico despite their geographical distances.

We have determined that for both species of *Scaevola* half of the populations showed allelic variation in the microsatellite regions analyzed, suggesting that there is higher genetic diversity within populations rather than between groups for each species. Because genetic variation resides inside isolated populations, rather than across populations, the loss of any single population of the native species would mean a loss in genetic diversity overall. Given the threat of dispersal by the invasive congener, our recommendation is the management and control of *S. taccada* to preserve existing genetic variations of *S. plumieri*.

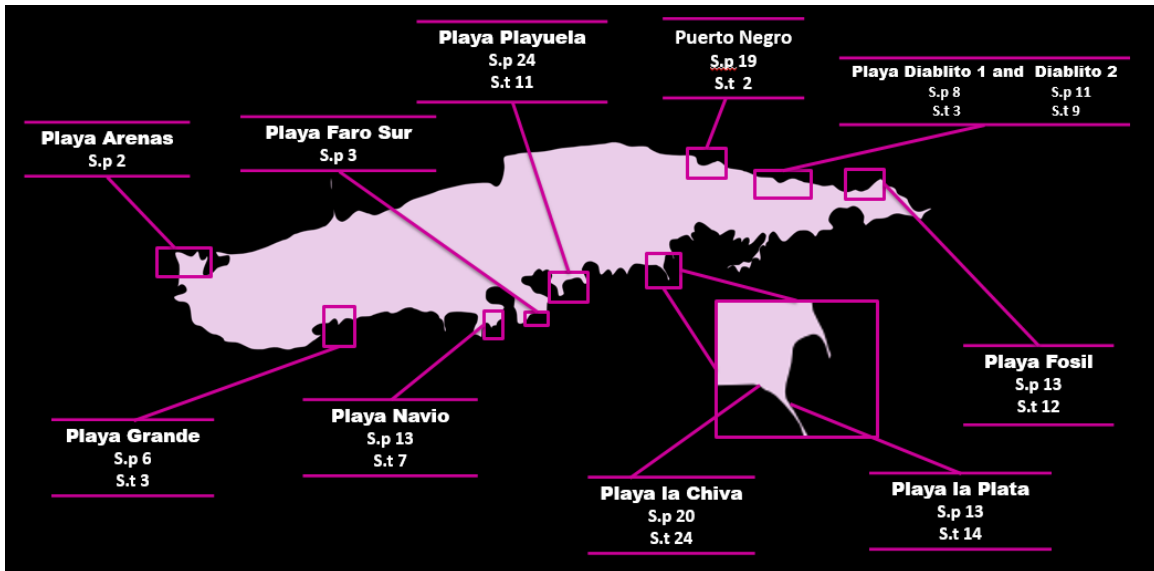


Figure 1. Map locations of sample collected in Vieques, Puerto Rico. In every location either *Scaevola plumieri* (*S.p*) or *Scaevola taccada* (*S.t*) was collected during winter break of 2015, 2016 and 2017.

References

- [1] Garcia-Verdigo, C. et al. (2015) Do island plant populations really have lower genetic variation than mainland populations? Effects of selection and distribution range on genetic diversity estimates. *Molecular Ecology*, 24, 726-741.
- [2] Allendorf, F., Luikart, G. and Aitken, S. (2013) *Conservation and the Genetics of Populations*. Wiley-Blackwell.
- [3] Breckon, G.J. (2007) Report on the Flora of Vieques Island, Puerto Rico. University of Puerto Rico, Mayaguez.
- [4] Finkle, A.J. and Elliot, N.B (2011) Status of endemic *Scaevola plumieri* and *Scaevola taccada* on San Salvador Island, Bahamas. The 13th symposium on the Natural History of the Bahamas, 46-51.
- [5] McCaffrey, K.T. (2009) Fish, Wildlife and Bombs: The Struggle to Clean up Vieques. *NACLA Report on the Americas*, 42(5).
- [6] Selkoe, K. and Toonen, R. (2006) Microsatellite from ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9: 615-629.
- [7] Ando, H., Emura, N., Denda, T., Nakahama, N., Inoue-Murayama, M. and Isagi, Y. (2014) Development of microsatellite markers for the coastal shrub *Scaevola taccada* (Goodeniaceae). *Applications in Plant Biology*: 2(5).
- [8] Shi, M., Guo, X., Zhou, L. and Zhang D. (2014) Isolation and characterization of sixteen polymorphic microsatellite loci for *Scaevola taccada* (Goodeniaceae), a widespread coastal plant. *Journal of Genetics*: Vol 93.
- [9] Wulff, A., Hollingsworth P., et al. (2012) Ten nuclear microsatellite markers cross-amplifying in *Scaevola montana* and *S. coccinea* (Goodeniaceae), a locally common and a narrow endemic plant species of ultramafic scrublands in New Caledonia. *Conservation Genet Resour*, 4:725-728.
- [10] Excoffier, L. and H.E. L. Lischer (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*. 10: 564-567.