



Landscape and Conservation Genetics of the Island Oak, *Quercus tomentella*

Mary V. Ashley*, Janet R. Backs, and Saji T. Abraham
Department of Biological Sciences, University of Illinois at Chicago
Chicago, IL 60607, USA

*corresponding author: ashley@uic.edu

ABSTRACT

The island oak, *Quercus tomentella* Engelm., is a rare island endemic, found only on five California Channel Islands and Guadalupe Island, Mexico. *Quercus tomentella* is a member of section *Protobalanus* that until recently had uncertain evolutionary origins and affinities. The most widespread species of *Protobalanus*, *Q. chrysolepis* Liebm., is found on the mainland but also on some of the islands and may hybridize with *Q. tomentella*. Here we present the first population genetic survey of *Q. tomentella* using DNA microsatellites analysis. A total of 378 trees were sampled from several sites on each of the islands where *Q. tomentella* occurs to assess levels of genetic diversity and to determine how that diversity is partitioned within and among islands. Genotypes were also used to quantify the extent of clonal versus sexual reproduction in *Q. tomentella*. Cryptic clonal growth was extensive at some sites. We found that *Q. tomentella* maintains moderate levels of genetic diversity despite having small, isolated populations. Populations on each island are genetically distinct, and significant population differentiation also occurs within islands. Such strong local population structure is in contrast to other studies of oaks, where high gene flow through pollen generally keeps populations homogeneous over large areas.

Keywords: microsatellites, California Islands, conservation genetics, clonal reproduction, island endemic, population structure, *Protobalanus*



Figure 1/ The range of *Quercus tomentella*. Arrows indicate islands where *Q. tomentella* occurs and where samples for this study were collected.

Introduction

The island oak, *Quercus tomentella* Engelm., is an island endemic, found only on five of the eight California Channel Islands (Anacapa, Santa Rosa, Santa Cruz, Santa Catalina and San Clemente) and on Guadalupe Island, off the coast of Baja Mexico (Figure 1). With such a limited distribution, it is certainly a rare oak and the rarest oak in the state of California. It is listed as vulnerable on the IUCN Red List of endangered species and is considered critically imperiled in Mexico. Because of its vulnerability and because it represents an important and distinct component of California Channel Island biodiversity, we undertook the first population genetics study of *Q. tomentella* throughout its range. Our objectives were to better understand the diversity of and relationships among the populations of *Q. tomentella* within and among islands.

Geological history

The uplift of the California Channel Islands, where *Q. tomentella* occurs, began over five million years ago (Atwater 1998; Schumann et al. 2012). Some of the islands lie fairly close to the mainland (Anacapa is only 20 km from mainland California), but there has never been a land bridge connecting the islands to the mainland, even during periods of lower sea level (Junger and Johnson 1980; Vedder and Howell 1980). As a result, the flora and fauna of the islands represent a rather depauperate subset of species found on



Photo 1/ Santa Catalina Island.

the mainland, descended from those species that successfully colonized the islands via overwater dispersal. There are a fair number of endemic species on the islands, including *Q. tomentella*, although most have relatively close affinities to mainland species. Although the islands were never connected to the mainland, they were connected to each other during periods of sea-level lowering. As recently as 18,000 years ago, the four northern islands (Santa Rosa, Santa Cruz, San Miguel and Anacapa) formed a single large island known as Santarosae.

Human presence and impact

The first human residents of the islands were Native Americans, arriving more than 10,000 years ago. The Chumash occupied the Northern Channel Islands and the Tongva/Gabrieleno people inhabited the Southern Channel Islands. Both were sophisticated maritime cultures that relied primarily on marine resources but also likely affected the natural plant communities. Their marine travels facilitated exchange of goods, possibly including acorns, which were a major food source of mainland Native Americans in California (Arnold 1992). Europeans began settling the islands in the mid-19th century, bringing with them non-native herbivores (sheep, goats, pigs, and others) that subsequently decimated much of the island vegetation, including oak woodlands (Westman 1983; Knowlton et al. 2007). Recent efforts to remove these herbivores have been successful, and the recovery of native tree and shrub communities is evident (Beltran et al. 2014; Rick et al. 2014). Currently the most vulnerable population is on Guadalupe Island, where only a small population survives.



Phylogeny

Quercus tomentella belongs to section *Protobalanus*, the Intermediate or Golden Oaks, a small group of oaks restricted to the Southwestern United States and Northwestern Mexico. The most widespread species of section *Protobalanus*, *Q. chrysolepis* Liebm., is found on the mainland but also on some of the islands where it may hybridize with *Q. tomentella*.

Photo 2/ Multiple stems of a tree on Santa Catalina Island. Leaf samples collected from five different stems were genetically identical, confirming that this is a single clone.

The evolutionary affinities of section *Protobalanus* have been long debated and uncertain, but a recent phylogenetic study using sequenced RAD data (Hipp et al. 2014) reports good support for *Protobalanus* (represented by *Q. chrysolepis*) being more closely related to the American White Oaks (section *Quercus*) than to the Red Oaks (section *Lobatae*). *Quercus tomentella* is often reported to be a relict species; a similar species, *Q. declinata* E. Dorf, is represented in late Tertiary fossil floras of the California mainland.

We were interested in answering three questions about *Q. tomentella*: 1) Is there evidence for reduced genetic variability in this island endemic? 2) Are populations on different islands connected by gene flow, or does each island have a genetically distinct population? 3) Is there evidence of genetic structure within islands?

Methods

To answer these questions, we collected samples from 378 trees from the six islands where *Q. tomentella* occurs. Our sampling included between 4 and 197 samples per island (Table 1), and we tried to spread sampling across each island, although limited accessibility prevented sampling some areas. We used previously published DNA microsatellite loci developed for *Quercus* to score each individual's multilocus genotype (Ashley et al. 2010; Abraham et al. 2011).

While sampling on Santa Catalina Island, we noticed that at some sites, the stems of *Q. tomentella* were growing in circular clumps. We suspected that these were clones, so for three of these clumps, we collected leaves from five different stems to test for clonal growth. In each case, their multi-locus genotypes were identical across all eight loci tested. The probability of this happening by chance through sexual reproduction, rather than because of cloning, is less than 10^{-7} , thus confirming clonal growth. More surprising were results from another site on Santa Catalina where the trees were not growing in clumps and cloning was not suspected. At a site called Lone Tree Grove, we found only 2 genotypes among 14 trees sampled (Ashley et al. 2010), suggesting that clonal growth might be extensive for *Q. tomentella*. However, among all the islands we found 296 genotypes among the 378 individuals genotyped, indicating that most reproduction occurred through acorns (Table 1). For our subsequent analysis, we considered only genetically unique individuals.

Island	N	Unique Genotypes	Avg. # alleles/locus	H _E
Santa Rosa	197	124	9.5	0.654
Santa Catalina	75	64	10.1	0.773
Anacapa	4	4	2.25	0.356
Santa Cruz	23	21	4.9	0.571
Guadalupe	23	22	7.6	0.669
San Clemente	56	55	9.6	0.752
Overall	378	290	7.33	0.629

Table 1/ Sample sizes (N) and descriptive statistics for *Quercus tomentella* genotyped at eight polymorphic microsatellite loci by island and overall samples. H_E is the expected heterozygosity.

We found that levels of genetic variation of *Q. tomentella* were quite typical for other oaks (Table 1), including widespread mainland species. For example, the average number of alleles per locus was over 10 on Santa Catalina, the island with the most genetic diversity, and expected heterozygosity (H_E) was 0.773. It was encouraging that the small remaining population on Guadalupe also had relatively high genotypic diversity, allelic diversity, and heterozygosity. Overall the levels of diversity suggest that the populations have not undergone a recent or prolonged genetic bottleneck, and that the populations have genetically recovered from founder effects that might have been associated with colonization of the islands by small numbers of propagules.

To look for genetic structure within and among islands, we took two approaches. Measures such as Wrights F_{ST} or Nei's genetic distance are standard measures used to evaluate how genetically different two or more populations are from each other. The values for these two measures are shown in Table 2 for comparisons between pairs of islands. The values are relatively large, and all were significantly different than zero (P < 0.01). Beyond that, Guadalupe Island tended to be genetically more differentiated from the other islands, which is not surprising since it is the most remote island. Other measures were somewhat unexpected because they did not seem to reflect geographic distance between the islands. For example the F_{ST} distance for the neighboring islands of Santa Rosa and Santa Cruz (0.049) was slightly more than the distance between Santa Rosa and Santa Catalina (0.041), and only slightly less than the distance from Santa Rosa to San Clemente (0.058), a very distant island (Table 2, top row). These findings suggest that something other than isolation-by-distance has influenced the population genetic structure of *Q. tomentella*.

	Santa Rosa	Catalina	Anacapa	S Cruz	Guadalupe	S Clemente
Santa Rosa	--	0.041	0.156	0.049	0.125	0.058
Catalina	0.201	--	0.162	0.057	0.073	0.037
Anacapa	0.423	0.477	--	0.192	0.223	0.180
S Cruz	0.198	0.243	0.520	--	0.132	0.053
Guadalupe	0.686	0.455	0.849	0.608	--	0.082
S Clemente	0.305	0.290	0.603	0.209	0.507	--

Table 2/ Genetic Distances Among Islands: F_{ST} above diagonal, Nei's Genetic Distance below. All values are statistically significant (p<0.05).

Another approach for evaluating the genetic relationships among populations is to look for genetic clusters within the genetic data set, irrespective of the population to which individuals are previously assigned (in this case to islands). One approach, called STRUCTURE (Pritchard et al. 2000; Hubisz et al. 2009) uses Bayesian analysis to assign individuals to genetic clusters based only on their genotypes and posterior probabilities. Our STRUCTURE analysis suggested that there were three genetic clusters within *Q. tomentella* (Figure 2). Most trees from Santa Rosa, where we had the largest number of samples (197) were assigned to a “northern island” cluster shown in blue in Figure 2. All trees from Guadalupe Island and most trees from San Clemente Island were assigned to a “southern island” cluster shown in orange in Figure 2. Trees from Santa Cruz Island, Anacapa Island, and most trees from Santa Catalina Island showed mixed northern and southern ancestry. However, one small group of trees on Santa Catalina Island were genetically quite distinct from all other *Q. tomentella* sampled, and formed their own genetic cluster which is shown in purple in Figure 2. Since those trees were collected at a site where *Q. chrysolepis* was also reported to be growing, these trees warrant further investigation as either being misidentified or introgressed with genes from *Q. chrysolepis*.

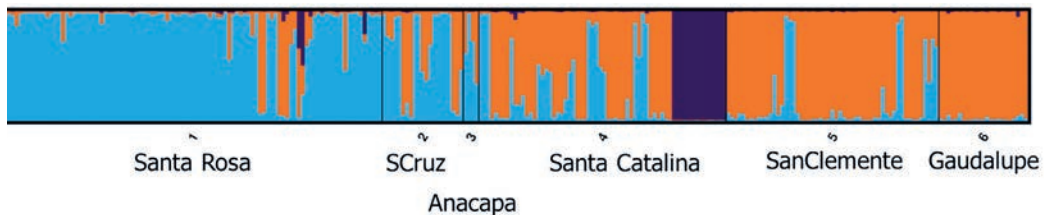


Figure 2/ Results of STRUCTURE analysis. Three genetic clusters were found, shown here in blue, orange and purple. The proportion of the membership coefficient in each cluster for individuals from each island are shown in vertical bars. Note the group of trees from Santa Catalina Island that comprise a distinct purple cluster.

The final analysis we undertook, to investigate genetic structure in *Q. tomentella*, was implemented in the software GENELAND (Guillot et al. 2005). Like STRUCTURE, it uses Bayesian analysis to assign individuals to genetic clusters, but includes both the genotype information and the spatial location of individuals. GENELAND identified 14 genetic clusters within the *Q. tomentella* samples. Each island had one or more unique genetic cluster, that is, none of the genetic clusters were shared among islands. Anacapa, Guadalupe Island, and San Clemente Island each had their own genetic cluster. Multiple clusters were found on Santa Rosa Island (3), Santa Cruz Island (2), and Santa Catalina Island (6). Most studies of mainland species of oaks show little or no genetic structure over large geographic areas and studies of pollination in oaks have revealed that average pollination distances may be hundreds of meters (Ashley 2010), and even isolated stands receive a large proportion of pollen (~50%) from trees outside of the stand (Craft and Ashley 2010; Buschbom et al. 2011). Thus the existence of genetically differentiated populations among stands of oaks occurring in relatively close proximity on one island was an unexpected result.

Discussion

What could account for such striking genetic structure within an island? It is difficult to say, but here we will take liberty with some speculation. It does not seem likely that



Photo 3/ *Quercus tomentella* on Santa Catalina Island.

topographic barriers such as mountains could account for these patterns, since they do not generally limit gene flow in mainland oak species. Differences in flowering phenology would be worth investigating, because asynchronous flowering (not common in oaks) could create a barrier to gene flow even over short geographic distances. A third possibility is that the original human inhabitants of the islands inadvertently or intentionally established genetically differentiated stands of *Q. tomentella* by transporting acorns among islands or between the islands and the mainland. As mentioned above, the Chumash and Tongva/Gabrieleno inhabited the islands for thousands of years, and navigated between the islands and the mainland using plank canoes called *tomols*. Although archaeological excavations suggest that acorns were not a major trade item among the islands (Fauvelle 2013), even limited exchanges could have resulted in establishment of genetically distinct stands of trees, especially like those found on Santa Catalina Island.

Conclusion

In conclusion, our investigation of microsatellite genotypes in the island oak, *Q. tomentella*, found little evidence for reduced genetic variability for this rare island endemic. This bodes well for conservation efforts, especially now as oak woodlands on the islands recover from effects of overgrazing. The highest genetic diversity was found on Santa Catalina Island, but even the critically imperiled Guadalupe Island population harbors sufficient diversity to recover if its habitat can be protected. We found that different islands have genetically distinct populations of *Q. tomentella*, suggesting that gene flow between islands is limited. As a result, each island harbors unique genetic variation and all islands merit protection. Genetically differentiated populations were found on some islands, especially Santa Catalina, and we speculate that movement of acorns by Native Americans may have played a role in shaping the current genetic structure of island oaks.

Acknowledgments

We thank the following organizations for facilitating this research: The Channel Islands National Park, the Catalina Island Conservancy, Grupo de Ecología y Conservación de Islas, and San Clemente Island Native Habitat Restoration Program. We thank Laura Kindsvater, Denise Knapp, Luciana Luna, Kevin Rice, Ian Pearse, Alfonso Munoz, Kathryn McEachern, Dirk Rodriguez, Florence Caplow, Kim Klementowski and Kate Faulkner for collecting or facilitating the collection of samples.

Photographers. Title page: Béatrice Chassé (*Quercus tomentella* on Santa Cruz Island). Photos 1-3: Mary V. Ashley.

Works cited

- Abraham, S.T., D.N. Zaya, W.D. Koenig, and M.V. Ashley. 2011. Interspecific and intraspecific pollination patterns of valley oak, *Quercus lobata*, in a mixed stand in coastal central California. *International Journal of Plant Sciences* 172: 691-699.
- Arnold, J.E. 1992. Complex Hunter-Gatherer-Fishers of Prehistoric California: Chiefs, Specialists, and Maritime Adaptations of the Channel Islands. *American Antiquity* 57: 60-84.
- Ashley, M.V. 2010. Plant parentage, pollination, and dispersal: how DNA microsatellites have altered the landscape. *Critical Reviews in Plant Sciences* 29: 148-161.
- Ashley, M.V., S. Abraham, L.C. Kindsvater, D.A. Knapp, and K. Craft. 2010. Population structure and genetic variation of island oak, *Quercus tomentella* Engelm on Santa Catalina Island. In *Oak ecosystem restoration on Santa Catalina Island, California: Proceedings of an on-island workshop, February 2-7, 2007* edited by D.A. Knapp. Avalon, CA: Catalina Island Conservancy.
- Atwater, T. M. 1998. Plate tectonic history of southern California with emphasis on the western Transverse Ranges and northern Channel Islands, 1-8. In *Contributions to the geology of the Northern Channel Islands, Southern California*. American Association of Petroleum Geologists, Pacific Section.
- Beltran, R.S., N. Kreidler, D.H. Van Vuren, S.A. Morrison, E.S. Zavaleta, K. Newton, B.R. Tershy, and D.A. Croll. 2014. Passive recovery of vegetation after herbivore eradication on Santa Cruz Island, California. *Restoration Ecology* 22: 790-797.
- Buschbom, J., Y. Yanbaev, and B. Degen. 2011. Efficient Long-Distance Gene Flow into an Isolated Relict Oak Stand. *Journal of Heredity* 102: 464-472.
- Craft, K.J. and M.V. Ashley. 2010. Pollen-mediated gene flow in isolated and continuous stands of bur oak, *Quercus macrocarpa* (Fagaceae). *American Journal of Botany* 97: 1999-2006.
- Fauvel, M. 2013. Evaluating cross-channel Exchange in the Santa Barbara region: Experimental data on acorn processing and transport. *American Antiquity* 78: 790-798.
- Guillot, G., F. Mortier, and A. Estoup. 2005. GENELAND: a computer package for landscape genetics. *Molecular Ecology Resources* 5: 712-715.
- Hipp, A.L., D.A. Eaton, J. Cavender-Bares, E. Fitzek, R. Nipper, and P.S. Manos. 2014. A framework phylogeny of the American oak clade based on sequenced RAD data. *PloS one* 9:e93975.
- Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* 9: 1322-1332.
- Junger, A., and D. L. Johnson. 1980. Was there a Quaternary land bridge to the northern Channel Islands?, 33-39. In *The California Islands: Proceedings of a multidisciplinary symposium*. Santa Barbara Museum of Natural History.
- Knowlton, J.L., C. Josh Donlan, G.W. Roemer, A. Samaniego-Herrera, B.S. Keitt, B. Wood, A. Aguirre-Muñoz, K.R. Faulkner, and B.R. Tershy. 2007. Eradication of non-native mammals and the status of insular mammals on the California Channel Islands, USA, and Pacific Baja California Peninsula Islands, Mexico. *The Southwestern Naturalist* 52: 528-540.
- Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
- Rick, T.C., T.S. Sillett, C.K. Ghalambor, C.A. Hofman, K. Ralls, R.S. Anderson, C.L. Boser, T.J. Braje, D.R. Cayan, and R.T. Chesser. 2014. Ecological Change on California's Channel Islands from the Pleistocene to the Anthropocene. *BioScience* 64: 680-692.
- Schumann, R.R., S.A. Minor, D.R. Muhs, L.T. Groves, and J.P. McGeehin. 2012. Tectonic influences on the preservation of marine terraces: Old and new evidence from Santa Catalina Island, California. *Geomorphology* 179: 208-224.
- Vedder, J.G. and D.G. Howell. 1980. Topographic evolution of the southern California borderland during late Cenozoic time, 7-32. In *The California Islands: Proceedings of a multidisciplinary symposium*. Santa Barbara, California: Santa Barbara Museum of Natural History.
- Westman, W.E. 1983. Island Biogeography: Studies on the xeric shrublands of the Inner Channel Islands, California. *Journal of Biogeography* 10: 97-118.