Largest Known *Quercus garryana* Douglas ex Hook Clone Discovered on a Steep Slope at the Boundary of Larrabee State Park, Washington, USA

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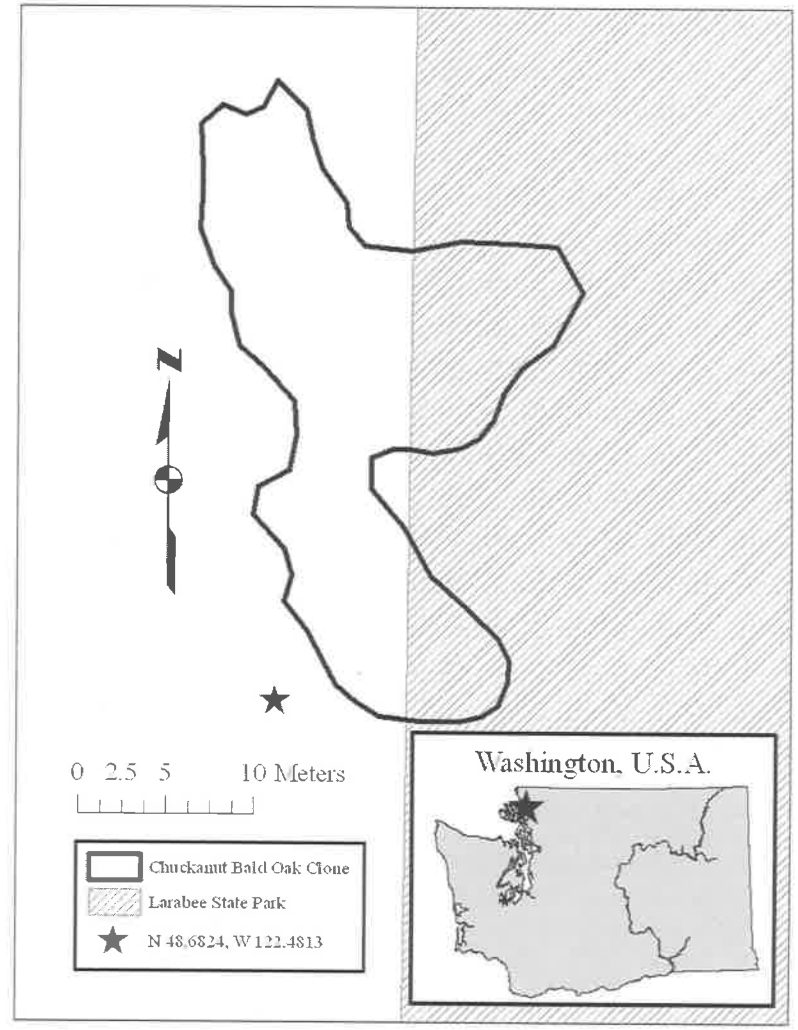
Abstract

The occurrence of clonal growth in deciduous trees is fairly common, particularly for regeneration after stem damage. Within the genus *Quercus* there are many species that have been described as possessing the ability to reproduce vegetatively, but the discovery of large clones is limited. Here we describe the largest known clone of *Quercus garryana* Douglas ex Hook. produced by extensive suckering. The *Q. garryana* clone described is located on a steep, west-facing slope at the boundary of Larrabee State Park, Washington, USA. Twenty-eight twig samples within the stand were collected and processed for genetic analysis. The samples were analyzed using seven nuclear microsatellites, and the results showed that all individual samples were genetically identical. The combined canopy of this clone covers an estimated 383m2 and is ~37m long (N – S) and ~20m wide (W – E) at its largest dimensions. In this case, we propose that the mechanism of continual cloning is a result of the perception of fallen stems due to gravitational pull on a steep slope. It is not yet known if this stand of *Q. garryana* is one of many large clones or unique, but it highlights the need for research into the mechanisms driving clonal growth to understand rates of vegetative biomass accumulation and possible trade-offs between sexual and asexual reproduction in woody plants.

Introduction

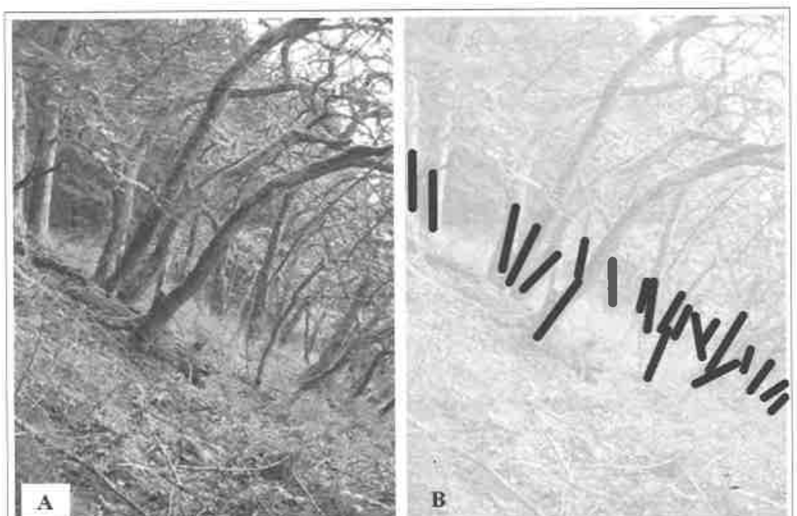
Some tree species that reproduce vegetatively through clonal growth produce very large clones, including the largest recorded organisms on Earth. For example, the 'Pando' clone of quaking aspen (*Populus tremuloides* Michx.) holds the record for the single largest organism at over 40 ha (100 acres) and an estimated 6 million kg (13.2 million lb) (Grant *et al.* 1992, Grant 1993, DeWoody *et al.* 2008). Other tree species, however, reproduce vegetatively by basal sprouting and root suckering when disturbed (Koop 1987, Jeník 1994, Jensen and Anderson 1995). Clonal clusters of deciduous trees often represent a few genetically identical trunks produced after stem damage from logging, coppicing, fire, wind, flooding, or browsing by herbivores (Roy 1955, Barsoum *et al.* 2004, Valbuena-Carabaña *et al.* 2008). Clonal formation of oak ramets is thought to be more akin to stump



Outline of *Quercus garryana* clone at western boundary of Larrabee State Park, Washington, USA. The star indicates GPS coordinate taken to mark the edge of the clone. Also shown is an inset map of the State of Washington, with the clone GPS coordinate depicted in the northwestern corner of the state.

sprouting or suckering after stem damage than as continuous underground spreading by genets as observed with aspen (Tiedemann *et al.* 1987, Guerin 1993, Sugihara *et al.* 1987, Ainsworth *et al.* 2003, Valbuena-Carabaña *et al.* 2008). Even for oak species that are known to be the most prolific clonal growers, clones

Leafless oak stems at top and right of figure. photo©Derrick Parker



A partial view of the *Quercus garryana* clone sample site and surrounding vegetation (A), and the same image with 21 of the largest visible *Q. garryana* ramets highlighted for improved visualization of oak stems in the stand (B). photo©Derrick Parker

are typically small with coverage areas less than 100m^2 (Montalvo *et al.* 1997, Alfonso-Corrado *et al.* 2004), though at least one species, *Quercus havardii* Rydb., has a documented individual estimated at 7,000m^2 (Mayes *et al.* 1998).

Quercus garryana Douglas ex Hook. (Garry oak or Oregon white oak) can grow as a large, broad-crowned, single-trunked tree, but it is also known as a stump-sprouting and cluster-forming oak species adapted to regular low-intensity fire regimes (Sudworth 1908, Roy 1955, Sugihara *et al.* 1987, Engber *et al.* 2011). In this paper, we describe a large clone of *Q. garryana* that occurs on a steep west-facing slope at the boundary of Larrabee State Park, Washington, USA. Using genetic techniques described in Marsico *et al.* (2009), we were able to identify the ramets of this clone as being genetically identical. The clone described here is the largest known (in canopy area) clone of *Q. garryana*. Determining if this clone is unique or if large clones are commonplace in *Q. garryana* is important to understanding drivers of growth form, rates of growth, and potential trade-offs in sexual and asexual reproduction.

Methods

The *Q. garryana* clone described is located on Chuckanut Bald along the western boundary and near the northern end of Larrabee State Park in Washington, USA (Fig. 1; Duemmel 2004). The site is located on a steep west-facing hillside (Fig. 2) overlooking Bellingham Bay and Lummi Island and is located on the eastern edge of a bald surrounded primarily by coniferous forest at an elevation of 292m. Twenty-eight twig samples were collected from throughout the stand on 5 April 2007, and they were dried individually in plastic bags on silica gel. Details of genomic DNA extraction, primer optimization, PCR, and fragment analysis can be found in Marsico *et al.* (2009). Briefly, Marsico *et al.* (2009) utilized seven nuclear microsatellite markers (quru-GA-0C19, quru-AC-0G12, quru-GA-0M05, quru-GA-1G13, quru-GA-1M17, ssQpZAG 36, ssQpZAG 9) to investigate population genetic structure across the northern half of the *Q. garryana* species range. In sampling 334 individuals at 22 sites from southern Oregon, USA, to British Columbia, Canada, only three pairs of identical individuals were found using these markers (Marsico *et al.* 2009). This indicates that these nuclear microsatellites are sensitive to slight genotypic variation within oak populations, making them appropriate for identifying genetically identical plants.

GPS coordinates were projected in ArcMap 9.3 (Esri, Redlands, CA) using the NAD 1983 reference system for Washington State Plane North and overlaid with a Larrabee State Park Boundary layer (WDNR 2007). A USGS 7.5 Orthoimage (USGS 2011) was georeferenced and compared with field notes to create a digitized Chuckanut Bald oak clone canopy coverage (Fig. 1). Using the ArcMap geometry calculator, the area was calculated for the oak clone canopy coverage. The USGS 7.5 minute Bellingham South Quadrangle was used to calculate percent slope by dividing the change in elevation by the change in horizontal distance. The degree of slope was calculated using the arctangent of change in elevation divided by change in horizontal distance over an area containing the clone.

Young shoots emerge from the root collar and stem base of older stems, showing the continued clonal growth in this stand. photo©Derrick Parker

Results and Discussion

Our genetic analysis of microsatellite markers showed that the individual twigs sampled at Chuckanut Bald had identical genotypes (Table 1). Of the 28 samples collected, 21 provided complete and identical genotypes with the markers. The other seven samples were consistent with the 21 complete samples,

but they did not amplify PCR product at one or two of the genetic markers. Small sprouts from the larger trunk bases, root collars, and lateral root systems were observed (Fig. 3), along with many larger stems comprising the stand (Fig. 4). The clone covers an area approximately 37m long and 20m wide at the longest and widest points, with a total estimated canopy area of 383m^2 (Fig. 1).

The large, continuously expanding *Q. garryana* clone we identified appears to be the largest recorded naturally occurring clone of this species. Interestingly, the clone seems to be continually spreading and producing new shoots in response to a disturbance perceived by the larger stems in the stand. Even though the most common mechanisms leading to clonal oak stands are fire, logging, coppicing, and/or over-grazing (Roy 1955, Sugihara *et al.* 1987, Montalvo *et al.* 1997, Bakker *et al.* 2001), this site has no indication of these events recently, though new sprouts were discovered in 2007 and appear to be produced annually. We propose that this stand of *Q. garryana* continues to expand due to the perception of fallen trunks caused by the gravitational pull down the steep slope (*i.e.*, 36% grade or 19.8°; see Fig. 2). This clonal mechanism has been observed for trees that have partially or completely fallen over and remain alive (Koop 1987, Jeník 1994), though it has not previously been documented for *Q. garryana* at this scale of regeneration. Therefore, due to specific environmental conditions—the steep slope, in this case—the Chuckanut Bald *Q. garryana* clone may be unique, though certainly these oaks also grow on very steep slopes in other locations. Alternatively, it is possible that large *Q. garryana* clones are relatively common but have simply not been recognized. *Quercus garryana* is known to have high allelic diversity within its populations (Marsico *et al.* 2009), but even in species known for clonal growth, genetic diversity at the population level remains high (Mayes *et al.* 1998, Alfonso-Corrado *et al.* 2004), making population genetic diversity a poor predictor of clone formation. Therefore, it is reasonable to speculate that large clonal stands of *Q. garryana* may simply have been overlooked. Further study of large clones is important to elucidate strategies of energy investment in clonal plants because trees stimulated to grow in large clones may accumulate biomass more rapidly and partition resources differently than non-clonal organisms (Mock *et al.* 2008).

Table 1. Shared nuclear microsatellite genotype of 28 *Quercus garryana* stems at Chuckanut Bald, Larrabee State Park, Washington, USA. Genetic data obtained using seven nuclear microsatellite markers following Marsico *et al.* (2009)

Nuclear microsatellites	0C19	0G12	0M05	1G13	1M17	ZAG 36	ZAG 9
Q. garryana Chuckanut	223	208	194	178	113	210	252
Bald clonal genotype	229	217	206	178	117	214	252

Acknowledgements

The authors would like to thank Jill Deines, Derrick Parker, and Kirsten Prior for their assistance in the field. Jim Duemmel originally sampled a subset of twigs that led us to investigate the possibility of a large clone. He also guided us to the site to make additional collections. The Romero-Severson lab at the

University of Notre Dame allowed use of equipment for sample preparation and microsatellite analysis. This work was supported by the Office of Science (BER), US Department of Energy Grant DEFG02-05ER. Derrick Parker took all photos used as figures in the manuscript.

References

Ainsworth, E. A., Tranel, P. J., Drake, B. G., Long, S. P. 2003. The clonal structure of *Quercus geminata* revealed by conserved microsatellite loci. Molecular Ecology 12: 527-532.

Alfonso-Corrado, C., Esteban-Jiménez, R., Clark-Tapia, R., Piñero, D., Campos, J. E., Mendoza, A. 2004. Clonal and genetic structure of two Mexican oaks: *Quercus eduardii* and *Quercus potosina* (Fagaceae). Evolutionary Ecology 18: 585-599. doi: 10.1007/s10682-004-5145-5.

Bakker, E. G., Van Dam, B. C., Van Eck, H. J., Jacobsen, E. 2001. The description of clones of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. with microsatellites and AFLP in and ancient woodland. Plant Biology 3: 616-621.

Barsoum, N., Muller, E., Skot, L. 2004. Variations in levels of clonality among *Populus nigra* L. stands of different ages. Evolutionary Ecology 18: 601-624. doi: 10.1007/s10682-004-5146-4.

DeWoody, J., Rowe, C. A., Hipkins, V. D., Mock, K. E. 2008. "Pando" lives: Molecular genetic evidence of a giant aspen clone in central Utah. Western North American Naturalist 64: 493-497. doi: : http://dx.doi.org/10.3398/1527-0904-68.4.493.

Duemmel, J. 2004. Native plants in native places: Our Garry oaks. The Potent Teller, December issue: 4.

Engber, E. A., Varner III, J. M., Arguello, L. A. Sugihara, N. G. 2011. The effects of conifer encroachment and overstory structure on fuels and fire in an oak woodland landscape. Fire Ecology 7: 32-50. doi: 10.4996/fireecology.0702032.

Grant, M. C. 1993. The trembling giant. Discover Magazine October issue. Accessed online 22 November 2011 at http://discovermagazine.com/1993/oct/thetremblinggian285.

Grant, M. C., Mitton, J. B., Linhart, Y. B. 1992. Even larger organisms. Nature 360: 216.

Guerin, D. N. 1993. Oak dome clonal structure and fire ecology in a Florida longleaf pine dominated community. Bulletin of the Torrey Botanical Club 120: 107-114.

Jeník, J. 1994. Clonal growth in woody plants: A review. Folia Geobotanica & Phytotaxonomica 29: 291-306.

Jensen, E. C., Anderson, D. J. 1995. The reproductive ecology of broadleaved trees and shrubs: Glossary. Forest Research Laboratory, Oregon State University, Corvallis. Research Publication 9f. 8 p.

Koop, H. 1987. Vegetative reproduction of trees in some European natural forests. Vegetatio 72: 103-110.

Marsico, T. D., Hellmann, J. J., Romero-Severson, J. 2009. Patterns of seed dispersal and pollen flow in *Quercus garryana* (Fagaceae) following post-glacial climatic changes. Journal of Biogeography 36: 929-941. doi: 10.1111/j.1365-2699.2008.02049.x.

Mayes, S. G., McGinley, M. A., Werth, C. R. 1998. Clonal population structure and genetic variation in sand-shinnery oak, *Quercus havardii* (Fagaceae). American Journal of Botany 85: 1609-1617.

Mock, K. E., Rowe, C. A., Hooten, M. B., DeWoody, J., Hipkins, V. D. 2008. Clonal dynamics in western North American aspen (Populus tremuloides). Molecular Ecology 17: 4827-4844.

Montalvo, A. M., Conard, S. G., Conkle, M. T., Hodgskiss, P. D. 1997. Population structure, genetic diversity, and clone formation in *Quercus chrysolepis* (Fagaceae). American Journal of Botany 84: 1553-1564.

Roy, D. F. 1955. Hardwood sprout measurements in northwestern California. USDA Forest Service, Forest Research Notes 95. California Forest and Range Experiment Station, Berkeley. 6 p.

Sudworth, G. B. 1908. Forest trees of the Pacific slope. United States Department of Agriculture, Washington, DC. 441 p.

Sugihara, N. G., Reed, L. J. 1987. Prescribed fire for restoration and maintenance of Bald Hills oak woodlands. pages 446-451 in Plumb, T. R., Pillsbury, N. H., tech. cords. General Technical Report PSW-100. Pacific Southwest Forest and Range Experiment Station, Berkeley, CA. 462 p.

Tiedemann, A. R., Clary, W. P., Barbour, R. J. 1987. Underground systems of Gambel oak (*Quercus gambelii*) in central Utah. American Journal of Botany 74: 1065-1071.

USGS. 2011. Bellingham South Quadrangle, Washington. 7.5 minute series. 1:24,000. [Portable Document Format]. United States Geological Survey, United States Department of Interior, Washington, D.C. Accessed at: http://store.usgs.gov/ on November 21, 2011.

Valbuena-Carabaña, M., González-Martínez, S. C., Gil, L. 2008. Coppice forests and genetic diversity: A case study in *Quercus* pyrenaica Willd. from central Spain. Forest Ecology and Management 254: 225-232.

WDNR (Washington Department of Natural ResourcesWDNR). 2007. Washington State Non-DNR Major Public Lands. [digital vector data]. Washington Department of Natural Resources. Accessed at: http://fortress.wa.gov/dnr/app1/dataweb/dmmatrix.html#Cadastre%20Jurisdiction%20 Boundaries on November 21, 2011.