

Clonal Growth and Fine-Scale Genetic Structure in Tanoak (*Notholithocarpus densiflorus*: Fagaceae)

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Abstract

The combination of sprouting and reproduction by seed can have important consequences on fine-scale spatial distribution of genetic structure (SGS). SGS is an important consideration for species' restoration because it determines the minimum distance among seed trees to maximize genetic diversity while not prejudicing locally adapted genotypes. Local environmental conditions can be expected to influence levels of clonal spread and SGS, particularly in the case of disturbance regimes such as fire. Here, we characterize fine-scale genetic structure and clonal spread in tanoak from drier upland sites and more mesic lowland woodlands. Clonal spread was a significant mode of stand development, but spread was limited on average to about 5–6 m. Gene dispersal was decomposed into clonal and sexual components. The latter varied according to whether it was estimated from all ramets with the clonal component removed or for a single ramet per genet. We used the difference in these 2 estimates of gene dispersal as a measure of the effect of clonality on effective population size in this species. Although upland sites had a greater number of ramets per genet, most of the other indices computed were not significantly different. However, they tended to show greater heterozygote excess and shorter gene dispersal distances than the lowland sites. The average distance among inferred sibships on upland sites was approximately at the scale of maximum clonal range. This was not the case on lowland sites, where sibs were more dispersed. We recommend minimum distances among seed trees to avoid selecting clones and to maximize genetic diversity for restoration.

Key words: *clonal growth, effective population size, gene dispersal, genetic diversity, Notholithocarpus densiflorus, restoration*

An overall goal of habitat restoration following severe disturbances is to maximize genetic diversity through appropriate management of recruitment from natural or artificial seeding. The choice of seed is critically important to avoid practices that would reduce evolutionary potential of the rehabilitated habitat and should be based on knowledge of patterns of genetic diversity at several spatial scales from that of the total species' range down to the local population. At the local scale, demic structure develops as a result of locally restricted gene dispersal and genetic drift (Wright 1977; Vekemans and Hardy 2004). The size of local demes and their associated variances of gene dispersal can be used to infer the minimum distances among trees for seed collection and to promote genetic diversity in the seed stock while not prejudicing locally adapted gene complexes. Many woody plants combine reproduction by seed with some

level of asexual spread and the latter may have important consequences on spatial genetic structure (SGS), particularly at the local level. Restricted gene dispersal arises not only from seed and pollen, but also, from spatial displacement of vegetative units (Gliddon et al. 1987) that will be a function of the mode of vegetative spread. In long-lived woody plants, clonal spread is commonly by resprouting and the degree of clonal spread will depend on the origin of resprouts (basal burls, crowns and lignotubers, or roots) and on the number of generations of asexual spread. Therefore, the effects of sprouting on SGS are expected to be along a continuum depending on the mode of sprouting and the degree of post-sprout thinning, with basal sprouting presumably contributing least to axial gene dispersal and root sprouting contributing much more. Two further consequences of resprouting are local matings among

clonal members that will strengthen local SGS (Eckert 2000; Thompson et al. 2008), and in predominantly outcrossing species, increased SGS as a result of biparental inbreeding (Premoli and Steinke 2008).

Ecologists generally agree that populations that combine sprouting with opportunistic seeding benefit from persistence (the persistence niche of Bond and Midgley 2001) in the face of disturbance events, as well as recruitment that may introduce novel genotypes. For basal resprouting species, competition among ramets may result in persistent replacement or a relatively slow accumulation of ramets per genet through time. Superficially, such populations could be indistinguishable from those that arose entirely from seed recruitment. The challenge in natural populations is to disentangle the relative contributions of seed and sprouting, given that genets can persist for many generations (de Witte and Stöcklin 2010). Indirect approaches can be used to infer the likelihood that genets have been persistent through generations, provided that resprouting contributes to some degree of genet expansion. First, somatic mutations should slowly accumulate through time, with the result that slightly divergent genotypes may be inferred as potential clone mates providing their occurrence is spatially consistent (Alberto et al. 2005; Arnaud-Haond et al. 2007; Mock et al. 2008). Second, genet sizes and inter-ramet distances can provide indirect estimates of generations of replacement (Escaravage et al. 1998; Ally et al. 2008; de Witte and Stöcklin 2010).

Clonality in modern-day angiosperms appears to have evolved as a derived condition (Kelly 1995) in a range of environments from cold, nutrient poor, and low light (Van Groenendael et al. 1996) to those that are regularly disturbed, as under a strong fire regime (Lamont and Wiens 2003). Presumably, trade-offs between seeding and sprouting under a range of conditions can explain the balance between reproductive modes. Bellingham and Sparrow (2000) proposed that under high intensity disturbance, seeding should prevail over sprouting when disturbance is at low frequency because investment in the production of storage organs for sprouting would restrain growth. Trade-offs under increasing frequency of disturbance would favor sprouters because seeders would need higher relative growth rates and shorter time to seed production than the disturbance return period. Also, the accumulation of stored energy can promote the rapid sprouting response after fire. Ultimately, under very short disturbance intervals, seeding should again be advantageous but directed to change away from woody vegetation. This model implies that populations of a single species will vary in the relative contributions of seeding to sprouting according to local environmental conditions, as has been shown for fragmented *Quercus ilex* woodland (Ortego et al. 2010) and stands of *Nothofagus antarctica* spanning a precipitation gradient (Premoli and Steinke 2008). To advance our understanding of the relative roles of seeding and sprouting in ecosystem structure and dynamics, more information is needed on how populations vary under different environmental stresses. The focus at a population level allows, not only the characterization of reproductive mode, but also its effect on genetic diversity and SGS; both likely to be affected by levels of seeding

and sprouting and both affecting evolutionary outcomes as a function of environmental change or stasis.

Tanoak (*Notholithocarpus densiflorus* ([Hook. & Arn.] Manos, Cannon & S. Oh); syn: *Lithocarpus densiflorus* ([Hook. & Arn.] Rehder) is a forest tree species that sprouts prolifically from the root collar after severe disturbance and, although it also produces large quantities of viable seed, the success of seedlings is rather low (Tappeiner and McDonald 1984). Populations in northern coastal California occur in a fire-prone ecosystem where disturbance events are likely to have been more frequent on more xeric upland sites. Recently, tanoak has suffered severe mortality from sudden oak death disease caused by the pathogen *Phytophthora ramorum* (Rizzo et al. 2002; Rizzo and Garbelotto 2003) and restoration will be needed to rehabilitate sites with this keystone species. Detailed understanding of the fine-scale genetic structure, including extent of clones, is critically important for developing strategies for seed collection from local sources for restoration. Our goal here was to characterize fine-scale genetic structure and clonal spread in xeric upland sites and more mesic lowland woodlands. First, we anticipated that given the fire-prone ecosystem and the probable presence of tanoak in coastal California through at least the last glacial maximum (Nettel et al. 2009), successive events of resprouting should have led to most genets being comprised of multiple ramets. We expected that fine-scale genetic structure would develop not only from clonal aggregations, but also from limited gene dispersal associated with the species' reproductive biology. Tanoak has erect aments indicative of insect pollination, and preliminary inflorescence-bagging studies confirm that insect pollination is the predominant mode of pollination (Dodd unpublished data). Acorns are heavy and fall close to the parent tree or are dispersed principally by acorn-predatory rodents and birds (Kennedy 2005). Second, we expected that the degree of clonality and SGS would be greater on upland sites where the fire-return interval is likely to be more frequent. Tanoak occupies humid lowland sites, where it is commonly associated with redwood (*Sequoia sempervirens*), and on xeric upland sites, where it is associated with Douglas-fir (*Pseudotsuga menziesii*) and mixed evergreen woodland and scrub. Upland sites are more fire prone and here tanoak may form denser stands of small-stature trees. We anticipated that the shorter fire-return interval would lead to increased resprouting and the shorter stature trees would allow more of the resprouts to reach tree size.

Materials and Methods

Sample Sites

Tanoak trees were sampled from 3 upland and 3 lowland sites in Jackson State Demonstration Forest (JSDF), Mendocino County, California (Figure 1a). This is a coastal location close to the centre of the range of tanoak. Tanoaks at the JSDF sites regenerated after fire at dates ranging from 40 to 60 years ago and were suspected to have regenerated at least in part by resprouting. Our sampling objective was 1-ha sample plots, to obtain adequate sample sizes for spatial analyses. However,

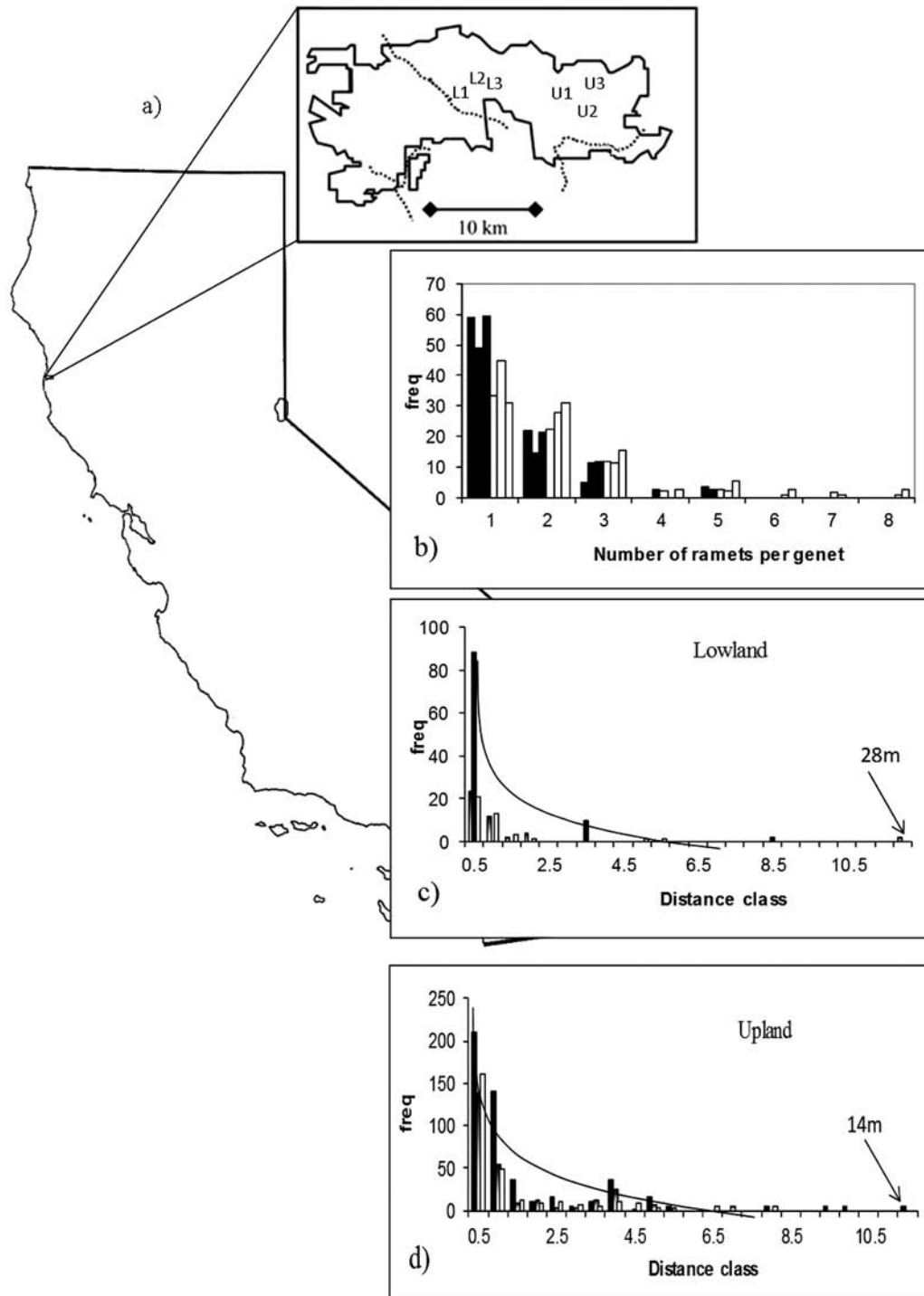


Figure 1. (a) Map of sample sites; (b) frequency distribution of numbers of ramets per genet (solid bars: lowland sites and unfilled bars: upland sites); (c) frequency distribution for clone mates within distance classes for lowland sites (grey bar site: 1, solid bar site: 2, and unfilled bar site: 3). Fitted logarithmic function $y = -13.8 \ln(x) + 22.8$; (d) frequency distribution for clone mates within distance classes for upland sites (solid bar site: 1, grey bar site: 2, and unfilled bar site: 3). Fitted logarithmic function $y = -39.9 \ln(x) + 74.5$.

at this location, we were unable to delimit plots of this size, in which tanoak was the dominant species, so we settled for two ½-ha rectangular plots at each of the lowland sites and two

¼-ha plots at each of the upland sites. Mean plant densities were 270 ramets/ha on lowland sites and 689 ramets/ha on upland sites (Table 1). On average, plots at each site were

Table 1 Sample sites and summary statistics for upland and lowland sites

	Lowland				Upland				Probability (lowland = upland)	F ratio
	L1	L2	L3	Mean	U1	U2	U3	Mean		
N	234	258	319	270.3	843	573	651	689.0	0.008	24.8
G	172	178	195	181.7	181	168	198	182.3	0.95	<0.01
G'	171	173	193	178.9	164	162	191	172.3	0.60	0.32
R	0.73	0.69	0.61	0.68	0.43	0.59	0.61	0.54	0.12	3.96
R'	0.73	0.67	0.61	0.67	0.39	0.57	0.60	0.51	0.11	4.09
CR	28.4	6.5	5.3	13.4	14.0	8.2	10.2	10.8	0.75	0.11
A	0.44***	0.61***	0.60***	0.55	0.565***	0.55***	0.53***	0.55	0.96	<0.01
H _e	0.54	0.58	0.55	0.56	0.61	0.56	0.58	0.58	0.23	2.00
R _t	3.7	3.8	4.0	3.83	4.0	4.0	4.1	4.03	0.10	4.50
F _{IS}	0.03	0.05	-0.04	0.01	-0.10*	-0.03	-0.08*	-0.07	0.07	5.90
S _{PR}	0.016*	0.043*	0.029*	0.029	0.049***	0.034***	0.038**	0.04	0.29	1.50
S _{PG}	0.006	0.016*	0.009*	0.01	0.010**	0.011**	0.009**	0.01	0.92	0.01
σ _{veg}	1.90	1.39	1.85	1.71	2.13	1.97	2.07	2.06	0.11	4.13
σ _{sex} ^a	27.8	16.7	21.3	21.9	14.8	15.4	13.5	14.6	0.09	5.08
σ _{sex} ^b	14.5	8.4	9.1	10.7	3.8	6.1	5.3	5.1	0.05	7.52
σ _{sex(Ne0.1)} ^a	87.8	52.8	67.3	69.3	46.9	48.7	42.7	46.1	0.09	5.07
σ _{sex(Ne0.1)} ^b	46.1	26.7	29.3	34.0	13.7	20.1	17.8	17.2	0.06	7.00
Δ _{ram} /δ _{R(cens)}	0.27	0.26	0.19	0.24	0.09	0.17	0.18	0.15	0.07	6.03

N: number of ramets per hectare; G: number of unique multilocus genotypes (MLGs); G': number of multilocus lineages (MLLs); R: genotypic diversity (based on MLGs); R': genotypic diversity (based on MLLs); CR: maximum clonal range; A: aggregation index; H_e: expected heterozygosity; R_t: allelic diversity (by rarefaction); F_{IS}: inbreeding coefficient; S_{PR}: index of spatial structure (Vekemans and Hardy 2004) based on all ramets; S_{PG}: index of spatial structure based on 1 ramet per genet; σ_{veg}: axial SD of clonal dispersal; σ_{sex}: axial SD of gene dispersal due to pollen and seed; δ_{ram}: calibrated density of ramets (see text); and δ_{R(cens)}: census density of ramets.

^aEstimates from genets.

^bEstimates from ramets; axial SD of dispersal based on census size except where indicated as one-tenth census size (N_{e0.1}).

F ratio based on 1 df between-groups and 4 df within-groups.

* Significant at $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$.

separated by 50 m. Ramets were exhaustively sampled in all plots and their spatial coordinates were obtained using a handheld Garmin Global Positioning System instrument. Stem diameters ranged from an average of 15.3 cm in the upland sites to 26.7 cm in the lowland sites. Fresh foliage was sampled for DNA analysis.

Nuclear DNA

Total genomic DNA was extracted from the fresh foliage using a simplified cetyltrimethyl ammonium bromide (CTAB) method (Cullings 1992). Eight nuclear microsatellite loci developed for tanoak (LD1, LD3, LD5, LD7, LD8, LD10, LD14, and LD17; Morris and Dodd 2006) were PCR amplified using a fluorescent-labeled primer. Primers were multiplexed in 3 groups: 1) LD1, LD3, LD7, LD10, LD14; 2) LD5, LD17; and 3) LD8. The PCR cocktail contained 1× PCR buffer, 2.0 mM MgCl₂, 0.2 mM of each dNTP, 250 nM of each reverse primer, 250 nM of each fluorescently labeled (FAM or HEX) forward primer, 1 unit of AmpliTaq polymerase (Invitrogen, Carlsbad, CA), and approximately 5 ng template DNA in a 20-μL reaction. Forward and reverse primers for the locus LD3 were run at a final concentration of 400 nM, exclusively. Touchdown PCR cycling conditions for all loci were as follows: 1 cycle at 95 °C for 10 min followed by 20 cycles of 45 s at 94 °C, 45 s at 58 °C (lowering 0.5 °C each cycle), and 45 s at 72 °C. The final amplification

step consisted of 20 cycles of 45 s at 94 °C, 45 s at 48 °C, and 45 s at 72 °C and was followed by a final extension step at 72 °C for 45 min. All reactions were performed on a Techne (UK) Flexigene thermocycler. We mixed 0.75 μL of PCR product with 8 μL of formamide and 0.5 μL of 500 LIZ size standard (Applied Biosystems, Foster City, CA), and we electrophoresed this cocktail on an ABI 3730 automated sequencer (Applied Biosystems). We used GENESCAN 3.7 and GENOTYPER 3.7 (Applied Biosystems) to analyze ABI results. We previously sequenced loci LD3 and LD5 because they showed odd-sized series in fragment analyses. The sequences showed that indels within the flanking regions were responsible for the different size series and so the fragment sizes were adjusted appropriately (Nettel et al. 2009).

Clone Identification

Clone mates were identified as individuals having the same multilocus genotype (MLG). Then, we assigned individuals as members of the same multilocus lineage (MLL) by treating pairs of MLGs with a single-step allele difference across loci as being ramets from the same genet that had accumulated a single mutation. All DNA samples in this category were then reamplified to check against amplification and scoring errors. The spatial proximity of these pairs of individuals was evaluated to estimate the probability that they belonged to MLLs *sensu* (Arnaud-Haond et al. 2007). For MLGs, we estimated

(P_{sex}), the probability that the same MLG could occur as a result of independent sexual reproduction events according to Parks and Werth (1993), adapted to take account of departures from Hardy–Weinberg equilibrium (HWE) after exclusion of multiple MLGs as described by Arnaud-Haond et al. (2007). Because of the multiple tests, we applied the Bonferroni correction for 12 independent tests, yielding an α value per test of 0.038.

Clonal Diversity and Spatial Aggregation of Clones

We estimated clonal diversity as genotypic richness using a modification of proportion distinguishable (Ellstrand and Roose 1987) to scale the parameter between 0 and 1 (Dorken and Eckert 2001):

$$R = \frac{G-1}{N-1}$$

where G is the number of MLGs (or MLLs) detected among N sampled ramets.

To assess the spatial organization of ramets, we estimated the spatial aggregation index (A), according to Arnaud-Haond et al. (2007). This index compares the average probability of clonal identity of all ramets in the stand with the average probability of identity among pairwise nearest neighbors; an index of 0 would indicate that clonal replicates are dispersed within the stand, whereas an index of 1 would indicate that clonal members are spatially aggregated. Also, we calculated the mean and maximum distance (CR) among identical MLGs and MLLs as a measure of the total spread of clonal patches.

All clonal identity, diversity, and spatial parameters were estimated using GenClone vers. 2.0 (Arnaud-Haond and Belkhir 2007). Variations among upland and lowland sites for clonal diversity indices were tested by analysis of variance after averaging plots within sites and treating the 3 upland and 3 lowland sites as independent replicates of site type.

Genetic Diversity and Inbreeding

For genetic diversity estimates, we used only single copies of an MLG. We estimated allelic richness with the rarefaction method (R_s) and expected heterozygosity (H_e) with FSTAT ver. 2.9.3.2 (Goudet 2001). To test for heterozygote excess, we assessed deviations from HWE within each site (by pooling genotypes from the 2 plots within each site) and for each locus as the inbreeding fixation index, F_{IS} , by randomizing alleles within samples 1000 times using the software FSTAT.

Spatial Genetic Structure

Spatial autocorrelation statistics are commonly used in studies of SGS. Here, we estimated the kinship coefficient of Loiselle et al. (1995) between pairs of individuals at a ramet and at a genet level and plotted the pairwise coefficients against fixed distance classes. Choice of distance class can affect autocorrelation plots and significance of relatedness in the distance classes. Because of high variance of relatedness coefficients, it is desirable

to have large numbers of pairwise comparisons that are evenly distributed in each distance class. To achieve this, we ran exploratory analyses in SPAGeDi v. 1.2 (Hardy and Vekemans 2002), with the number of distance classes set to 5, 8, 10, 12, and 15, but with undefined distance ranges, to inform us on the spatial partition that would optimize the numbers of pairwise comparisons per distance class and provide good spatial resolution. We tested 2, 5, and 8 m distance classes and present results here for 5 m. We performed analyses on all sampled individuals (ramets) and on single copies of MLGs (genets). For the latter, a single pair of central coordinates was computed from the spatial coordinates of all ramets per genet. Significance of spatial structure of genets or ramets was obtained by permuting sampling locations among pairwise sample units 10 000 times. Because spatial autocorrelations are dependent on the sampling scheme and genetic background of each sample plot, we did not formally test for differences among plots. Rather, we further described SGS by estimating S_p statistics described by Vekemans and Hardy (2004). The S_p statistic is given by

$$S_p = \frac{-b_F}{(1 - F_1)}$$

where, b_F is the linear slope of the pairwise kinship coefficient of Loiselle et al. (1995), for all pairs of individuals on the natural logarithm of the spatial distances among pairs of individuals. Vekemans and Hardy (2004) have shown that the slope of this regression divided by the complement of the mean coancestry coefficient for the first distance class (F_1) provides a statistically meaningful comparison of SGS among sample plots.

We evaluated the relative contributions of clonal growth and sexual reproduction to gene dispersal. The total variance of gene dispersal ($\sigma^2 = Nb/4\pi D$), where Nb is Wright's (1943) neighborhood size and D is the effective population density, can be separated into a clonal component (σ_{clon}^2) and a sexual component ($1/2\sigma_{\text{pollen}}^2 + \sigma_{\text{seed}}^2$) that includes the contribution of pollen and of seed (Gliddon et al. 1987). We calculated σ_{clon}^2 as the mean-squared distance between a ramet and the central coordinates of the clone to which it belongs. For genets with only 2 ramets, this was equivalent to one-half the mean-squared distance between the 2 ramets, and for genets with a single ramet, a dummy variable was created in which the distance to the central coordinates of the clone was set to 0.1 m (the minimum distance between ramets of a genet). The reciprocal of the S_p index, described earlier, provides an estimate of Nb , and the ramet-based estimate can be used to estimate the total variance in gene dispersal. We obtained our estimate for $\sigma_{\text{sex}}^2 = (1/2\sigma_{\text{pollen}}^2 + \sigma_{\text{seed}}^2)$ by subtracting (σ_{clon}^2) from the total variance in gene dispersal estimated from $\sigma^2 = [1/(4\pi S_p \delta)]$ (Vekemans and Hardy 2004), where δ is the effective density of ramets/m². We used 2 estimates of δ ; the first was census density from the sample plots and the second was one-tenth census density based on global average ratios of N/N_e reported by Frankham (1995). We recognize that estimates of effective population size are difficult to calculate in

established stands, where a patchy distribution of related seedlings may result from a seed decay kernel that does not follow a pattern of isolation by distance from maternal trees (Sezen et al. 2009). However, our purpose here was comparative; to investigate the overall effect of mixed sprouting and seeding on effective population size.

Relatedness

To further test spatial patterns of sibling occurrence at each site, we inferred full sibs, half sibs, and unrelated pairs of individuals using the “group-likelihood” approach implemented in COLONY (Wang and Santure 2009). In performing analyses, we assumed that male and female parents were polygamous and that the rate of genotyping errors was 1% and mutations and other errors were 3% (set to allow for a single mutation per locus consistent with our estimates for MLLs). We accepted only relationships with probabilities greater than 0.9.

Results

At all sampled sites, some MLGs were detected more than once. Probabilities that these identical genotypes could have arisen from independent sexual reproductive events were all less than 0.01, and so we interpreted these individuals as being clonal. In assigning MLLs, we found no inconsistencies in allele calling when samples with single-step allele differences among MLGs were reamplified, suggesting that genotyping error was not the cause. In all but 2 cases, samples differing by a single allele were in close spatial proximity. With the exception of these 2 spatially disjunct samples, single allele differences were treated as comprising MLLs. Only 8 individuals were detected with single allele differences (MLLs) on the lowland sites, contrasting with 30 samples that fell in the same category on the upland sites (Table 1). Genotypic richness (R) ranged from 0.43 to 0.73 for identical MLGs and from 0.39 to 0.73 for MLLs (Table 1).

Clonal Spatial Structure

Based on MLGs, most stems were identified as single ramet genets, but genets with 2 and 3 ramets were common and up to 8 ramets were detected on upland sites (Figure 1b). Although most ramets of multistem genets occurred within the 0.5 m distance class and the frequency of ramets at greater distances fell off rapidly, inter-ramet distances of about 5–6 m occurred across all upland sites and on one of the lowland sites (Figure 1c,d). We fitted logarithmic functions to the plot of frequency of ramets by distance class for all upland and lowland sites combined, shown in Figure 1c,d. Estimates of the distance class at which frequency reached 0, yielded 5.5 and 6.5 m for lowland and upland sites, respectively. These data suggest that multiple generations of tanoak resprouting had contributed to a gradual spatial spread of genets. The greatest distances between identical MLGs were 28 m on site L1 and 14 m on site U1 (Table 1). Although our estimate of P_{sex} (the probability of obtaining identical genotypes through

Table 2 Estimates of F_{IS} by locus and by population for upland (U) and lowland (L) populations

Locus	U1	U2	U3	L1	L2	L3
LD1	-0.25*	-0.09	0.01	0.09	0.18*	-0.14*
LD3	-0.22*	0.08	-0.10*	0.08	0.18*	-0.12*
LD5	-0.10	0.08	-0.18*	-0.11*	-0.03	0.05
LD7	-0.08	-0.15*	0.05	-0.15*	-0.06	0.06
LD8	-0.16*	-0.10*	-0.14*	0.12	0.02	-0.08
LD10	-0.18*	-0.07	0.08	0.05	-0.09	0.08
LD14	0.18*	-0.03	-0.20*	0.04	0.14*	-0.06
LD17	0.05	0.06	-0.18*	0.12*	0.08	-0.10*
Mean	-0.10*	-0.03	-0.08*	0.03	0.05	-0.04

Negative values denote heterozygote excess. Significance at the 95% levels are shown by asterisks.

sexual reproduction) was low, this probability increases with many pairwise comparisons, so that we cannot be confident that these widely separated individuals are clonal. The aggregation index ($\mathcal{A}$) that measures the degree of dispersal among ramets of a clone averaged 0.55 and was significant at all sites (Table 1). In a basally resprouting species, values for $\mathcal{A}$ would be expected to approach 1.0, if resprouting was limited to few generations as there would have been little opportunity for spatial spread.

Genetic Diversity

Genetic diversity assessed either as expected heterozygosity (H_e) and allelic richness (R_e) were not significantly different between sites (Table 1). Significant departures from HWE based on inbreeding coefficients (F_{IS}) were detected at some loci but not at others (Table 2). F_{IS} over all loci was significant at 2 of the upland sites, suggesting a possible trend for greater heterozygote excess on upland sites (Table 1).

Spatial Genetic Structure

Spatial autocorrelograms showed significant spatial structure in the shortest distance class for ramets at all sites (Figure 2). For genets, SGS was significant in the first distance class at all upland sites but only at one of the lowland sites. This first spatial class was capturing a large proportion of the clonal structure of the plots revealed by the greater difference between the ramet and genet kinship coefficients for this distance class than at any of the other distance classes. By the second distance class (5–10 m), kinship coefficients for genets were not significantly different from 0 at any of the sites. At all sites, spatial structure measured by the S_p statistic was greater with all ramets than with only single copies of genets included (Table 1). Although the S_p statistic for genets alone was greater in the upland sites, differences between sites were not significant. Axial standard deviations (SD) of clonal dispersion were short (Table 1), supporting the aggregation of clonal copies of each genet. Using the reciprocal of the S_p statistic as an estimate of Wright's neighborhood size, we obtained 2 estimates of σ_{sex} based on census population density and 2 estimates based on one-tenth census size: 1) based

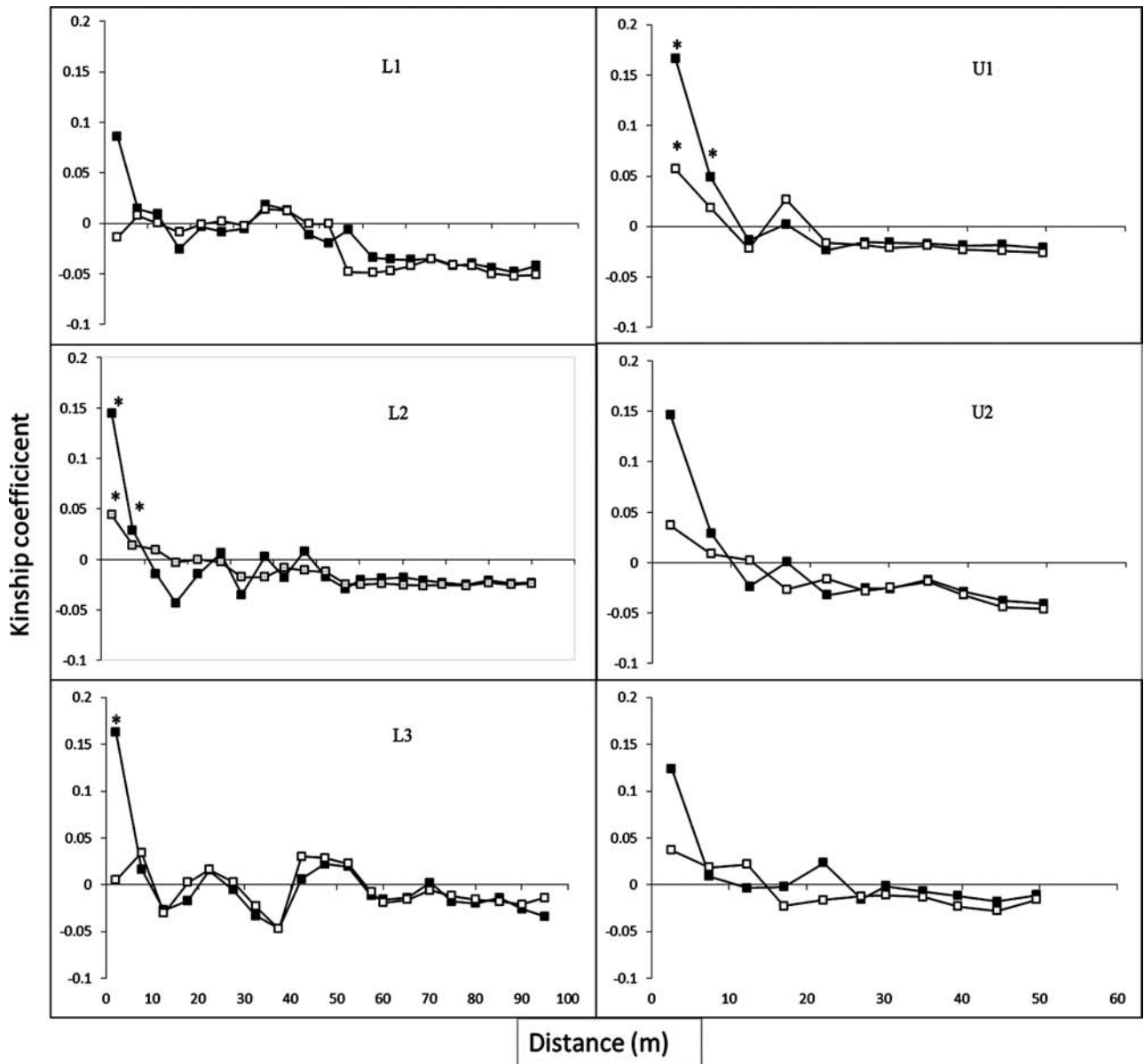


Figure 2. Spatial autocorrelograms based on Loiselle's kinship coefficients for 3 lowland and 3 upland sites (averaged over 2 subplots for each site). Asterisks indicate kinship coefficients significantly different from zero. Solid rectangles—all ramets, and open rectangles—single ramet for each genet.

on all ramets after subtracting axial variance in clonal dispersal and 2) based on genets. Estimates based on genets were about 2–3 times greater than those based on ramets, and estimates based on $N_{e0.1}$ were about 3 times greater than those based on census size (Table 1). Assuming the true values of σ_{sex} to be equal, whether estimated from ramets or genets, we calculated the density of ramets that would give the same value of σ_{sex} as that estimated from genets, by solving for δ_{ram} in the following equation:

$$\frac{Nb_{\text{gen}}}{4\pi\delta_{\text{gen}}} = \frac{Nb_{\text{ram}}}{4\pi\delta_{\text{ram}}} - \sigma_{\text{veg}}^2$$

This density was then expressed as a ratio of census density of ramets. The ratio ranged from 0.09 to 0.27, indicating that effective population size of populations combining seed reproduction and clonal spread is lower than in populations from seed alone.

The COLONY analysis detected an average of about 15% sibships among the genets, of which a small proportion (~1% on upland sites and 0.8% on lowland sites) were inferred as full sibs (Table 3). The proportions of full and half sibs did not vary between upland and lowland sites. The average spatial distance among full sibs was significantly less than half sibs on both upland and lowland sites. Presumably,

Table 3 Proportion of inferred sibships and mean intersib distances. Sibships estimated using COLONY (Wang and Santure 2009)

Site	Percentage of total genets		Mean intersib distance (m)	
	Full sibs	Half sibs	Full sibs	Half sibs
U1	1.14	13.9	6.0	11.2
U2	1.01	15.3	10.4	15.1
U3	0.98	14.8	9.6	12.9
Mean (SE)	1.04 (0.05)	14.67 (0.41)	8.67 (1.35)	13.07 (1.13)
L1	0.71	14.1	19.5	26.2
L2	0.82	12.4	25.5	30.2
L3	0.88	15.8	17.2	29.4
Mean (SE)	0.80 (0.05)	14.10 (0.98)	20.73 (2.47)	28.60 (1.22)

the greater dispersal distance of pollen over seed resulted in greater distances among paternal half sibs. Mean distances among full and half sibs were shorter on the upland sites (Table 3), contributing to their greater SGS.

Discussion

Spatial Clonal Structure

Two life-history traits are important in determining landscape patterns of clonality for a species: 1) frequency of asexual spread relative to seeding and 2) dispersal capability of asexual and sexual units. The former is likely to be affected by temporal and spatial environmental variations and the latter by species' asexual reproductive modes that range from seed of apomictic origin through a continuum of sprouting from rhizomes or roots to lignotubers and strictly basal.

Our analyses of MLGs indicated an average clonal richness of 0.61 based on MLGs over the 6 sampling sites. In other words, ~40% of the ramets in the stands were clonal replicates. When we allowed for potential somatic mutations that would give rise to genotypes with a single allele difference, clonal richness decreased only slightly to 0.59. These more inclusive MLLs were spatially consistent with the pattern of MLGs, and in all cases, the allelic difference was consistent with single-step mutations. Our inference of MLLs is conservative by limiting MLLs to one single-step mutation, thereby discounting additional mutational steps and multistep mutations. Furthermore, our detection of potential clone mates is limited to existing ramets, whereas if, as expected, genets had persisted over long periods of time, the ancestral seed-origin ramet would have been lost from the population and random mutations among descendent ramets would potentially produce a mosaic of ramets with mutations at different loci. We did not attempt to account for this, so our estimates of clonality are biased toward a strictly replacement mode for most genets that may significantly underestimate clonal spread (Ally et al. 2008).

Compared with studies of other sprouting species, our estimate of clonal richness was comparable with data from basal sprouting Fagaceae (Berg and Hamrick 1994; Montalvo

et al. 1997), in the fire-prone sprouter *N. antarctica* (Premoli and Steinke 2008), and European aspen (Suvanto and Latva-Karjanmaa 2005) and lower than that of root sprouters such as northern populations of *Betula verrucosa* (Hermanutz et al. 1989) and *Populus tremuloides* (Kemperman and Barnes 1976). However, it is noteworthy that, demographically, the tanoak system is not restricted to ramet replacement; plots of inter-ramet distances indicated a mean maximum distance of about 5–7 m over all sites (Figure 1c,d). The frequency distribution of ramets per clone (Figure 1b) could reflect several demographic processes within the stands including sexual recruitment over recent generations, local competition and fitness differentials among genets, and stochastic loss of ramets of some genets. We favor low-frequency seedling recruitment following a repeated recruitment model that has been shown to be common among species with short-distance seed dispersal (Eriksson 1989), as is likely to be the case for tanoak and was suggested as the cause for high genet diversity in *N. antarctica* (Premoli and Steinke 2008). It is unlikely that inter-genet competition plays a significant role because the distribution of genets is dependent on their pre-disturbance distribution that is likely to be well spaced.

Spatial Genetic Structure

We subdivided our estimates of SGS into clonal and sexual components. This is rarely considered in the literature on SGS in woody plants, yet many angiosperms combine asexual and sexual reproduction and the former can lead to elevated levels of SGS at short distances as demonstrated by autocorrelograms that compare all ramets with only genets from the same plot (Figure 2).

For tanoak, the average effect of clonality on SGS was limited (an overall average of about 2 m) as expected from our data on observed clonal structure. Our estimates of S_p for genets that represent a standardized measure of SGS, although low, were within the range reported for outcrossed tree species and were no different than average estimates for trees with animal pollination and heavy gravity- or animal-dispersed seeds (Vekemans and Hardy 2004). Low SGS in species with low seed vagility suggests a strongly outcrossing system that seems to be the case in insect-pollinated tanoak. Our preliminary data from progeny arrays indicate outcrossing rates in excess of 85% (Dodd unpublished data) that may not be significantly different from high outcrossing rates reported for wind-pollinated *Quercus* species (Schwarzmann and Gerhold 1991; Fernández-Manjarrés and Sork 2006). Furthermore, outcrossing rates commonly increase with age of tree cohorts. Estimates of gene dispersal distances (axial SD of gene dispersal) without the effect of clonality varied substantially according to the computational method. First, applying effective population density at one-tenth of census density yielded estimates of gene dispersal about 3 times greater than those based on census density. Census density is inappropriate for decomposing the composite parameter Nb and, although one-tenth census density has been suggested as an average factor for adjusting census size to effective

population size over a range of organismal groups (Frankham 1995), it can provide only an approximate estimate. Second, estimates based on a single ramet per genet were 2–3 times greater than those based on all ramets with axial variance in clonal dispersal removed. Theoretical expectations are that the 2 approaches should provide equivalent estimates of σ and differences between the 2 estimates are likely, at least in part, to be a result of the choice of ramet as a single copy of the genet and the effects of clonality on N_e (Orive 1993). The first of these is unlikely to have an important effect unless we have severely underestimated the extent of clonality due to “ghost” ramets or somatic mutations. On the other hand, we have shown that the choice of effective population size is important and could be much lower in clonal plants than in plants reproducing only sexually. We inferred the effect of clonality on effective population density by taking advantage of the 2 methods of deriving S_p and subsequently N_b , from all ramets and from genets only, by calculating the density of ramets that would give the same value of σ_{sex} as that estimated from genets alone. This value, referred to here as δ_{ram} , is independent of whether calculations are based on census density or on effective density estimated as a fraction of census density, in our case, one-tenth census density. Here, we calculated δ_{ram} from census numbers and expressed δ_{ram} as a proportion of census ramet density. Values ranged from 0.27 to 0.09, indicating that clonality in these tanoak stands reduced even further the effective population size by 10–25%. Accurate estimates of effective population sizes are notoriously difficult to obtain and our data illustrate that combined clonality and sexual reproduction can further confound the relationship between census size and effective population size. Furthermore, Wright’s N_b assumes dispersal–drift equilibrium, but it is very likely that the disturbance regime in these tanoak woodlands as a result of periodic fires, and because of human activities over the last ~150 years and even longer (taking into account activities by native Americans), has prevented these populations from reaching an equilibrium state.

Comparison of Upland and Lowland Sites

Stand density was greater and stem diameters narrower on the upland sites. We expected to find greater numbers of ramets per genet and increased SGS on these upland sites as was reported for *N. antarctica* (Premoli and Steinke 2008), and the data though nonsignificant tend to support this. One of the difficulties in such comparisons is identifying sites that are truly representative of the extremes. In most cases, site variation is more or less continuous and this probably contributed to the lack of significant differences in our data. Nevertheless, average number of ramets per clone tended to be greater on upland than on lowland sites resulting in somewhat reduced (but not significant) clonal diversity ($R = 0.54$ upland; $R = 0.68$ lowland). We anticipate that fire regimes are likely to be more frequent on the drier upland sites, which could result in a higher turnover of generations promoting clonality over seed reproduction. Although we

found no evidence of greater SGS as measured by S_p on the upland sites, gene dispersal distances were at the limit of significance, suggesting shorter gene dispersal due to seed or pollen and distances among full and half sibs (8.7 and 13.1 m, respectively on upland sites; 20.7 and 28.6 m, respectively on lowland sites) were shorter on the upland sites. This is unlikely to be purely a function of scale (denser stands in the upland localities) since the average spatial distance among sibs was equivalent to the maximum clonal range ($CR = 10.8$ m) on upland sites but was almost twice as great on the lowland sites ($CR = 13.4$ m). It is most likely that any seedling recruitment that does occur is at a more local scale on upland sites, perhaps as a result of shorter distance seed dispersal of the heavy acorns. We suggest that seedling recruitment is greater in the lowland sites as a result of reduced disturbance, however, more intense sampling is required to confirm this.

Conclusions

Mortality among tanoaks due to sudden oak death disease has reached epidemic proportions in some populations and restoration of sites is a high priority to minimize catastrophic fires and soil erosion. We do not propose that restoration should attempt to mimic site genetic structure but rather that maximum genetic diversity with minimum risk of planting maladapted genotypes would favor seed collections from nonclonal and unrelated individuals from as short a distance as possible from the site to be restored. To achieve this, in lowland sites, seed trees should preferably be selected from as diverse an array of individuals as possible, with a minimum of 20 m (maximum intraclonal spatial distance observed) between trees as a rule to avoid clonal replicates and greater than 70 m (based on gene dispersal estimates; Table 1) to maximize the likelihood of collecting seed from unrelated parents. On upland sites, distances among seed trees can be reduced with a minimum of 15 m likely to avoid clone mates and a minimum of 50 m to obtain seed from unrelated individuals.

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