New Phytologist

Research

No evidence of population structure across three isolated subpopulations of *Russula brevipes* in an oak/pine woodland

Sarah E. Bergemann[1], Greg W. Douhan[2], Matteo Garbelotto[1] and Steven L. Miller[3]

[1]University of California, Berkeley, Department of Environmental Science, Policy and Management, Division of Ecosystem Science, 137 Mulford Hall #3114, Berkeley, CA 94720, USA; [2]University of California, Riverside, Department of Plant Pathology, Riverside CA 92521, USA; [3]University of Wyoming, Department of Botany, 1000 University Ave, Laramie, WY 82071, USA

Author for correspondence:
Sarah E. Bergemann
Tel: +1 510 643 4282
Fax: +1 510 643 5436
E-mail: sbergemann@nature.berkeley.edu

Received: *3 September 2005*
Accepted: *23 November 2005*

Summary

• *Russula brevipes* is common ectomycorrhizal (EM) fungus that is associated with several hosts across temperate forest ecosystems. A previous study has demonstrated that substructuring across large geographic distances (1500 km) occurs in the western USA.

• To examine genetic structure over a more localized scale, basidiocarps of *Russula brevipes* from three subpopulations, separated by distances of 230–1090 m, were collected over two consecutive years in an oak/pine woodland. Microsatellite loci were used to test for population differentiation both among subpopulations and by year.

• No significant population differentiation was detected between subpopulations (θ_{ST} = 0.01) or between years (θ_{ST} = 0.01). Most loci were consistent with a Hardy–Weinburg equilibrium and 82% of the genets between seasons from similar sampling localities constituted new genotypes.

• These results indicate that *R. brevipes* constitutes a randomly mating population with no genetic differentiation between locations or across successive fruiting seasons.

Key words: allele, dispersal, ectomycorrhiza (EM), gene flow, microsatellite.

New Phytologist (2006) **170**: 177–184

doi: 10.1111/j.1469-8137.2006.01654.x

Introduction

The symbiotic relationship between roots and fungi (mycorrhizas) is one of the most prevalent associations in all ecosystems. Ectomycorrhizal (EM) fungi are prominent mutualistic partners with temperate trees and they facilitate the trees' uptake of mineral and organic nutrients and water, in exchange for photosynthates from their host (Smith & Read, 1997). Despite their obvious ecological importance, little is still known about the population biology of most EM species, which is intrinsically tied to how forest ecosystems function and evolve. For example, we still do not understand many of the fundamental life history processes that can shape EM populations such as the importance of mating vs vegetative growth, the prevalence of colonization by monokaryotic or dikaryotic mycelia, the spread of EM fungi across landscapes, and the spatial and temporal scales at which root colonization occurs. The study of EM population genetics can provide insights into these important concepts of EM fungi, especially with the modern tools of molecular ecology and studies which have focussed on specific hypotheses concerning EM fungal biology.

The focus of most population studies of EM fungi has been on an examination of genets in local populations. Several studies have evaluated the number and size of genets (see Dahlberg, 2001; Redecker *et al.*, 2001). These studies have demonstrated that the size of the genets is highly variable, depending on the EM taxon sampled. For example, Bonello

et al. (1998) used single stranded conformation polymorphisms (SSCP) in a population of *Suillus pungens* and found that a single genet covered an area of at least 300 m^2. In contrast, Redecker *et al.* (2001) used amplified fragment length polymorphic (AFLP) markers to estimate the genet size of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita fracheti* and found these to be relatively small (1.1–9.3 m^2).

The size and density of genets may provide important insights into the reproductive biology of EM fungi. Repetitive sampling of genets in patches directly underneath fruitbodies have demonstrated that the genets of several EM fungi are short-lived, including *Suillus grevillei*, *Hebeloma cylindrosporum* and *Laccaria amethystina* (Gryta *et al.*, 1997; Gherbi *et al.*, 1999; Fiore-Donno & Martin, 2001; Zhou *et al.*, 2001b). Small, short-lived genets are indicative of EM populations that reproduce primarily by sexual reproduction via meispores (see Dahlberg, 2001; Redecker *et al.*, 2001). At the other extreme, long-lived perennial genets are characteristic of EM fungi that can spread via mycelial expansion from point sources. EM fungi that exhibit long-lived perennial genets include *Leccinum duriusculum*, *Suillus* spp. and *Xerocomus pruinatus* (Dahlberg, 2001; Fiore-Donno & Martin, 2001; Selosse, 2003). The high frequency of genets in local populations of *R. brevipes* and the presence of several basidiocarps formed by a common below-ground mycelium has been observed in different stands (Bergemann & Miller, 2002). One question that has not been addressed is of whether the allele frequencies in *R. brevipes* are correlated between successive fruiting seasons.

To date, relatively few studies have been published that have estimated genetic differentiation or compared the correlation between genetic differentiation and the distance between EM populations. In *Rhizopogon vinicolor*, *Suillus grevillei*, *Xerocomus chrysenteron* and *Laccaria amethystina*, no evidence of genetic structure between locations was found at geographic scales ranging from tens to thousands of meters (Gherbi *et al.*, 1999; Kretzer *et al.*, 2000, 2005; von Melzer & Rothe, 2000; Zhou *et al.*, 2001a), with moderate levels detected across distances around 5 km in *R. vesiculosus* (Kretzer *et al.*, 2005). In contrast, it has been shown that the geographic isolation of *Tricholoma* spp. and *R. brevipes* has led to a significant genetic differentiation across global and continental scales (Bergemann & Miller, 2002; Chapela & Garbelotto, 2004). For *R. brevipes* sampled in Douglas-fir, lodgepole pine and Sitka spruce stands in western North America, high estimates of genetic differentiation based on θ_{ST} and F_{ST} suggested that gene flow between subpopulations was limited (Bergemann & Miller, 2002; Bergemann, 2002). It was also concluded that hosts do not appear to act as significant barriers to gene flow; rather, populations are geographically structured across western North America.

The objective of this study was to sample subpopulations of *R. brevipes* in more localized oak/pine woodlands to attempt to resolve the distance at which gene flow could occur. Specifically, we wanted to test for population differentiation among subpopulations isolated by distances of 230–1090 m, and to make inferences on their reproductive biology based on analyses of microsatellite loci.

Methods and Materials

Study site and mapping

Basidiocarps of *Russula brevipes* were collected from three locations on the western slope of the California Sierra Nevada Mountains, approx. 96 km north-east of Sacramento at the University of California Sierra Foothill Research and Extension Center in Yuba county (39°17′39″ N, 121°28′94″ W) (Fig. 1). The vegetation is characteristic of the Sierra foothill oak woodlands and is dominated by blue oaks (*Quercus douglasii*) intermixed with interior live oaks (*Q. wislizenii*) and gray pine (*Pinus sabiniana*) (Griffin, 1977). In the oak/pine vegetation series of the western Sierra Nevada, an uneven distribution of trees creates a mosaic of open savanna and woodlands (Johnson *et al.*, 2002). Basidiocarps of *R. brevipes* were collected in three woodland locations, comprised of a

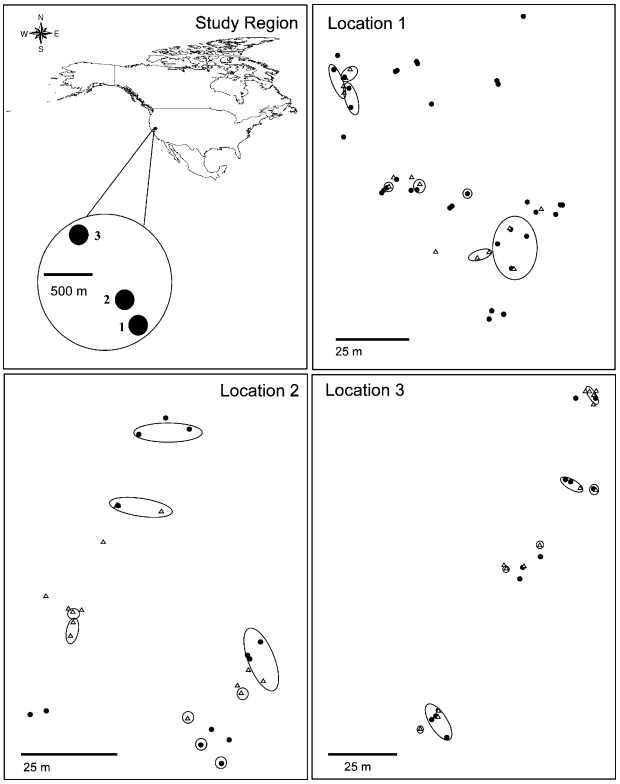

Fig. 1 Map of the study region in an oak/pine woodland showing the three locations of subpopulations of *Russula brevipes* and the relative positions of genets within each subpopulation. Closed circles, genets collected in 2003; triangles, genets collected in 2004; open circles, areas in which the same genet was mapped.

mixture of gray pine and interior live oak isolated by intervening meadows of perennial and annual grasses and forbs (Fig. 1). The positions of *R. brevipes* basidiocarps in two consecutive fruiting seasons (January, 2003–2004) were recorded using a Trimble GeoExplorer III Global Position Systems (GPS) hand-held unit (Sunnyvale, CA, USA). Once the positions had been recorded, the rover files were differentially corrected from the nearest base station to improve the accuracy of the measurements. After correction, maps were generated using ArcView GIS 3.2 (ESRI, Redlands, CA, USA) to estimate the distance between genets and locations.

DNA extraction and PCR amplification

Small amounts of tissue taken from the stipe trama were lyophilized and macerated by beating with 2 mm glass beads in a FastPrep machine (Qbiogene, Irvine, CA, USA). DNA from basidiocarps was extracted using a modified CTAB procedure as previously described (Bergemann & Miller, 2002). Six microsatellite loci were amplified using a PCR protocol that includes betaine and BSA additives with 'touch-down' cycling parameters allowing successive decrements in annealing temperatures (Bergemann *et al.*, 2005). Forward primers for each locus were labeled with HEX before PCR amplification. The detection of labeled PCR products was carried out on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the internal size-standard GeneScan-500 ROX for the determination of allele sizes. Allele sizes were estimated using GENESCAN (Applied Biosystems).

Representative PCR products from each locus were cloned to confirm the amplification of microsatellite repeats and to estimate the lengths of the flanking region and microsatellite repeats using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). PCR products were amplified using the M13 and T7 priming regions and the sequences were edited using Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI, USA).

Estimating allele frequencies, Hardy–Weinberg, and genotypic equilibrium

GENEPOP 3.4 (Raymond & Rousset, 1995) was used to calculate the frequency of alleles of *R. brevipes* for all locations (Table 1). Cervus 2.0 (Marshall *et al.*, 1998) was used to estimate the observed (H_O) and expected (H_E) heterozygosity by considering a 'population' as a location from a single year and after the inclusion of one genotype from a location (Table 1). To test for the presence of null alleles, observed genotypes were compared with a randomized distribution of alleles within each locus using Micro-Checker (van Oosterhout *et al.*, 2004). To determine the power of resolving genets, the observed allele frequencies of all basidiocarps were used to calculate the expectation of a genotype occurring by chance assuming random mating (Bergemann & Miller, 2002). Saturation between the number of alleles vs the number of basidiocarps sampled was plotted for each location to determine the estimated power of resolving allelic diversity in each subpopulation within a given year (Fig. 3).

Table 1 Expected and observed heterozygosities and the number of alleles and repeat length in subscripts (2 = di-repeat, 3 = tri-repeat) for six microsatellite loci of *Russula brevipes*

Locus	2003 No. of alleles	2004 No. of alleles	2003 H_O	2003 H_E	2004 H_O	2004 H_E
Location 3						
$RA5_2$	7	4	0.37*	0.63	0.44	0.55
$RA106_2$	3	2	0.70*	0.52	0.67	0.52
$RB23_3$	9	8	0.82	0.88	0.89	0.90
$RE14_2$	1	1	0.00	0.00	0.00	0.00
$RE102_2$	1	1	0.00	0.00	0.00	0.00
$RG1_3$	10	8	0.79	0.86	0.67*	0.88
Location 2						
$RA5_2$	3	5	0.66	0.54	0.15*	0.68
$RA106_2$	2	2	0.57	0.43	0.46	0.52
$RB23_3$	7	9	0.67*	0.86	0.92	0.85
$RE14_2$	1	1	0.00	0.00	0.00	0.00
$RE102_2$	1	1	0.00	0.00	0.00	0.00
$RG1_3$	7	7	0.78	0.88	0.69	0.77
Location 3						
$RA5_2$	4	5	0.12*	0.64	0.20*	0.66
$RA106_2$	2	2	0.67	0.47	0.50	0.40
$RB23_3$	7	8	1.00	0.85	1.00	0.84
$RE14_2$	1	1	0.00	0.00	0.00	0.00
$RE102_2$	1	1	0.00	0.00	0.00	0.00
$RG1_3$	10	9	0.89	0.91	0.80	0.88

*Significant departure from H-W equilibrium ($P < 0.05$).

Correlation measures of allelic diversity and estimates of genetic structure were performed to assess whether the allele frequencies from locations sampled in 2003 and 2004 were similar. A Spearman Rank Correlation coefficient was computed using SPSS 10.0 (Chicago, IL, USA) to determine whether the rank abundance of allele frequencies from 2003 and 2004 were correlated between sampling times. The percentage occurrence of alleles was calculated for each location using the equation: [(no. of times allele occurred in location)/(sample size × 2)] × 100. The correlation coefficient was computed for all paired locations across years using all loci, and once after removing any monomorphic loci that may have confounded the contribution of rare alleles.

Because *R. brevipes* is perennial and may form genets over successive years (Bergemann & Miller, 2002), departures from the gametic equilibrium may be as a result of an association between clones rather than loci (McDonald *et al.*, 1995); therefore, two datasets were compared: an uncorrected dataset that included all genets and a corrected dataset that included one representative of each genet within a location. Genotypic disequilibrium was tested using GENEPOP 3.4

with a Markov Chain algorithm for all pairs of loci for both corrected and uncorrected datasets (Raymond & Rousset, 1995).

Genetic structure

Genetic structure was estimated using a derivative of traditional measures (F_{ST}) of population differentiation with the corrected dataset (θ_{ST}, Weir & Cockerham, 1984) using GENEPOP 3.4 (Raymond & Rousset, 1995). Analysis of Molecular Variance (AMOVA) was used to partition the genetic variation to determine whether the genetic variance was better explained by the year sampled or by the locations of each subpopulation, using Arlequin 2.0 (Schneider *et al.*, 2000). A correlation between genetic distance (θ_{ST}) and geographic separation was computed for both genets and locations using GENEPOP 3.4 with the corrected dataset (Rousset, 1997; Rousset, 2000). First, the correlation between geographic distance and θ_{ST} was tested among locations, followed by correlations between θ_{ST} and genetic distance between genets.

Results

Allelic and genotypic diversity

The number of alleles ranged from one allele in two di-repeat loci (RE14, RE102) to 12 alleles in tri-repeat loci (RB23, RG1) (Table 1). In two loci (RE14, RG1), a small proportion (4.0% and < 1%, respectively) of the samples did not amplify with two PCR attempts. No evidence of null alleles was found, indicating that the nonamplification of alleles was likely to be a result of low template concentration or degraded DNA; therefore, no attempt was made to estimate the allele frequencies for these samples. The sequences of the amplified alleles of polymorphic loci confirmed the high frequency of the heterozygotes and the variation in allele sizes generally conformed to GENESCAN fragment lengths with the exception of one locus, RA5 (see Discussion below). GenBank accession numbers for the sequences are: RA5 (DQ107360-367), RA106 (DQ107368-371), RB23 (DQ107372-373), RE14 (DQ103374), RE102 (DQ107375-376) and RG1 (DQ107377-380).

Of the 124 basidiocarps sampled, 57 were sampled in location 1, 36 from location 2, and 32 from location 3 (Table 2). The total number of genets sampled within a single location over consecutive seasons ranged from 15 to 36 (Table 2, Fig. 1). Twelve genets were sampled in the same location over consecutive fruiting seasons: location 1 = 5, location 2 = 2 and location 3 = 4 (Table 2; Fig. 1). The expectation of encountering a genotype by chance was between 1.2×10^{-3} and 1.7×10^{-8}, demonstrating that the power of resolving genets was sufficient.

In all three locations sampled, significant correlations between allele frequencies in 2003 and 2004 were found (r_s = 0.75–0.92 ($P < 0.01$)) (Table 3, Fig. 2). Departures from a Hardy–Weinberg equilibrium were detected in four loci (RA5, RA106, RB23, RG1) (Table 1). In RA106, an excess of heterozygotes was detected in one location ($P = 0.03$) (Table 1). In contrast, a deficiency of heterozygotes in RB23 and RG1 were detected within two locations ($P < 0.05$) (Table 1). In RA5, several inserts and deletions in the flanking region of RA5 were found adjacent to the repeat region (data not shown). In this locus, variation of the GENESCAN fragment lengths because of both variation in the flanking sequence and repeat regions likely resulted in several observed heterozygote deficiencies (Table 1). Primers could not be re-designed to exclude the variable flanking region; therefore, the locus was eliminated from estimates of

Table 2 The number of *Russula brevipes* basidiocarps and the number of genets sampled

	Number of basidiocarps			Number of genets		
Location	2003		2004	2003		2004
1	38		19	31		10
Total		57			36 (5)	
2	14		22	10		12
Total		36			20 (2)	
3	13		19	9		10
Total		32			15 (4)	

Parentheses () refer to the number of genets sampled in two successive fruiting seasons (2003 and 2004).

Table 3 The computed Spearman Rank Correlation coefficient of the percentage of alleles sampled in locations of *Russula brevipes* in two consecutive fruiting seasons (2003 and 2004)

Location (Year)	1 (2003)	1 (2004)	2 (2003)	2 (2004)	3 (2003)	3 (2004)
1 (2003)	–	**0.90**	0.64	0.71	0.73	0.65
1 (2004)	**0.92**	–	0.57	0.63	0.64	0.65
2 (2003)	0.71	0.66	–	**0.73**	0.62	0.50
2 (2004)	0.77	0.70	**0.79**	–	0.70	0.65
3 (2003)	0.78	0.71	0.70	0.76	–	**0.81**
3 (2004)	0.72	0.72	0.66	0.72	**0.85**	–

The upper triangle of the matrix is the correlation coefficient between all locations after removing monomorphic loci (RE14 and RE102). Lower triangle shows rank correlation between locations with inclusion of five loci (RA106, RE102, RE14, RB23, RG1). Bold values signify the correlation made between the same location collected in 2003 and 2004. All correlation coefficients were significant ($P < 0.01$).

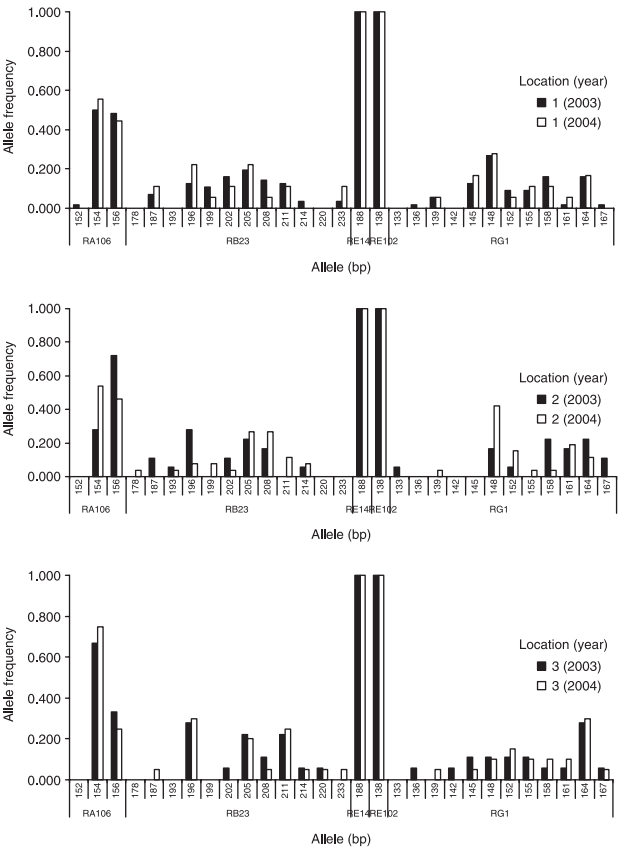

Fig. 2 The size (bp) and frequency of alleles of *Russula brevipes* sampled from basidiocarps from three locations in the western Sierra Nevada (USA) demonstrates that there is a high correlation between allele frequencies from two consecutive fruiting seasons (2003 and 2004).

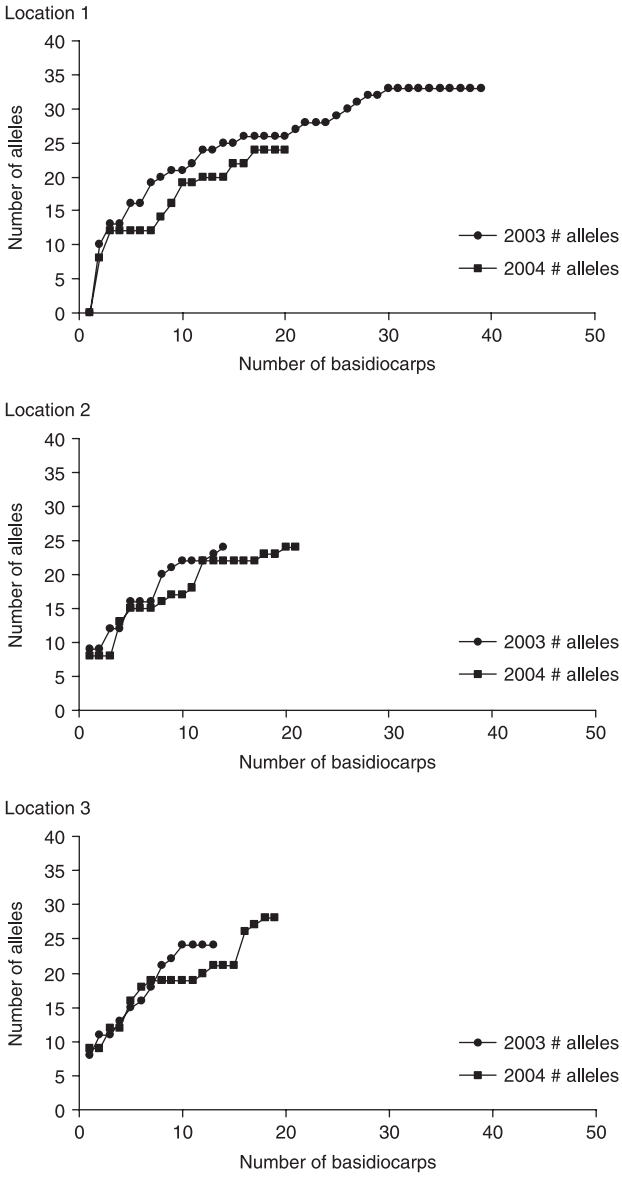

Fig. 3 The number of alleles of *Russula brevipes* plotted against the number of basidiocarps across two consecutive fruiting seasons (2003, 2004).

genetic structure, and genotypic equilibrium. After clone correcting and combining data from a single location, no departures from a Hardy–Weinberg distribution were detected ($P > 0.05$). Fourteen out of 17 tests of genotypic disequilibrium were significant when clone correction was not performed ($P < 0.05$), whereas no significant tests of genotypic disequilibrium were found using the corrected dataset ($P > 0.05$), suggesting that the absence of clone-correcting leads to associations between loci rather than to chromosome linkage.

Estimates of genetic structure

Analysis of Molecular Variance (AMOVA) demonstrated that most of the variance was explained by genetic diversity within locations, whereas an insignificant proportion of the variance was explained among years or locations (Table 4). Estimates of within-location structure across years indicated that there was little to no genetic structure between consecutive fruiting seasons (($\theta_{ST} = -0.03$ (location 1), $\theta_{ST} = 0.03$ (location 2), $\theta_{ST} = -0.04$ (location 3)) or among all locations across all years ($\theta_{ST} = 0.01$) (Table 5). When genets sampled in 2003 and 2004 from the same location were pooled and clone corrected, pair-wise estimates of genetic structure indicated little genetic differentiation within locations (($\theta_{ST} = 0.00$ (locations 1 & 2), $\theta_{ST} = 0.02$ (locations 1 & 3), $\theta_{ST} = 0.04$ (locations 2 & 3)) or among all locations ($\theta_{ST} = 0.01$).

Estimates of genetic structure (θ_{ST}) indicated a positive, insignificant correlation between genetic and geographic distance for three locations ($r_s = 0.66$; $P = 0.51$). There was no correlation between the genetic (θ_{ST}) and geographic separation of individuals within locations ((Location 1, $r_s = 0.05$

Table 4 Analysis of Molecular Variance (AMOVA) partitioning variation among groups of *Russula brevipes* (year or location sampled)

	Year			Location		
Source of variation	d.f.	Sum of squares	% of variation	d.f.	Sum of squares	% of variation
Among groups	1	1.1	−0.7	2	4.6	2.8
Among locations within groups	4	6.0	1.6	3	2.5	−1.1
Within locations	150	166	98	150	166	98
Total	155	166	99	155	166	99

Variation within the grouping variable (year or location) explained between 1.6% and 1.1% of the variance, respectively. The variance within locations explained between 98% of the variance.

Table 5 Estimates of population structure (θ_{ST}) of *Russula brevipes* within three locations sampled from two fruiting seasons (2003 and 2004)

Location (Year)	1 (2003)	1 (2004)	2 (2003)	2 (2004)	3 (2003)
1 (2004)	***−0.03***				
2 (2003)	0.02	0.01			
2 (2004)	0.01	0.00	***0.03***		
3 (2003)	0.00	−0.02	0.05	0.02	
3 (2004)	0.03	0.00	0.08	0.05	***−0.04***

θ_{ST} within locations sampled over successive fruiting seasons (2003–04) are shown in bold italics.

($P = 0.18$), Location 2, $r_s = 0.15$ ($P = 0.07$), Location 3, $r_s = 0.05$ ($P = 0.30$)).

Discussion

In previous studies of population differentiation among populations of *R. brevipes* from the west coast and Rocky Mountains, high estimates of genetic differentiation suggested that geographic barriers or distance between populations in western North America may have led to substantial genetic differentiation among populations; however, the absence of shared alleles indicated that populations from Wyoming and California may be cryptic species (Bergemann & Miller, 2002). In the current study, we dramatically decreased the spatial scale at which sampling was conducted and found that no isolation occurred between fungal subpopulations; rather, spore dispersal between sites has likely led to the observed homogeneity among the three locations separated by distances of 230–1090 m. This is also supported by the Hardy–Weinberg equilibrium model and by the recovery of new genotypes at each location coupled with a lack of allelic variation between years at each sampling subpopulation.

Another important aspect with respect to sampling is the adequacy of the sampling employed on a particular 'population', which will intrinsically be tied to the reproductive biology of the EM fungi studied. If the sampling effort using above-ground fruitbodies is not a reflection of the existence of diversity below-ground, then estimates of genetic structure may not reflect the population in question (Rogers & Rogers, 1999). For example, Selosse *et al.* (2001) evaluated how sampling could effect the interpretation of genet distribution of *Laccaria* spp. Since the fruiting of genets was not synchronized, it was suggested that a single sampling would have biased their estimates of genotypic diversity; therefore, an erroneous conclusion of low genet diversity would have been made, which was not the case when multiple sampling dates and years were considered (Selosse *et al.*, 2001). In the present study, several collections were made each year within a 3–4-week period, which covered most of the fruiting period for this species in the years sampled, since fruiting is highly correlated with rainfall in this Mediterranean ecosystem and varies dramatically by year (M. Smith, pers. comm.). Nevertheless, a more intensive sampling within years and over additional years would have likely resulted in the recovery of more genotypic diversity in this system and may have reinforced the absence of a genetic structure.

Our results support the idea that gene flow is occurring in *R. brevipes* in this ecosystem; however, the exact mechanism of its dispersal is unclear. It is generally assumed that wind is the predominant mechanism of spore dispersal of EM epigeous fungi, whereas animals facilitate spore dispersal in hypogeous EM fungi (Johnson, 1996; Molina *et al.*, 2001). In some studies it has been clear that spore dispersal has contributed to the high levels of genotypic diversity (Gherbi *et al.*, 2004); however, others have maintained that the wind gusts required at ground level may be inadequate for the dispersal of *Russula* spores (Allen & Hipps, 1984). In the field, we have observed that most basidiocarps of *R. brevipes* are subepigeous and

rarely mature above the litter layer, suggesting that wind may not be a primary mechanism for dispersal (Bergemann & Miller, 2002). In our present study, we found several remnants of basidiocarps that had been consumed by mammals such as small rodents or deer (S.E. Bergemann & G.W. Douhan, pers. obs.). We speculate that either animal-mediated dispersal (directly or indirectly) or wind dispersal may be factors in maintaining the absence of genetic structure in *R. brevipes* in localized populations. The range of foraging small mammals can approach more than 100 ha with a tendency to forage near EM trees, leading to an increased dispersal distance and likelihood of colonization (Johnson, 1996). However, further experimentation will be required to determine dispersal mechanisms in *R. brevipes*.

If aerial-dispersed spores are generally deposited in an exponential leptokurtic pattern whereby most spores land near the parental sporocarps (Gregory, 1945) and establish successfully, the relationship between geographic separation and the genetic distance of genets should be correlated. In this study, no correlation between the genetic and geographic separation of genets was found. Similar results were obtained in parentage analyses of *Rhizopogon vinicolor* and *R. vesiculosus*, whereby most genets sampled in immediate vicinities were unrelated (Kretzer *et al.*, 2005). Kretzer *et al.* (2005) hypothesized that competition may prevent the offspring of *R. vinicolor* and *R. vesiculosus* from establishing near parental genets. An alternative hypothesis to account for the incidence of unrelated genotypes in nearby locations is that mating systems strongly favor outcrossing, whereby spores from outside the resident subpopulation have a higher likelihood of successful mating and subsequent establishment. Gryta *et al.* (2000) found that the inbred progeny of *Hebeloma cylindrosporum* were often short-lived compared to the survival success of outcrossed mycelia. Elucidating the patterns of genet distribution in stands is likely to require a better understanding of the interactions operating between genets of EM fungi, an understanding of the longevity and residence of genets and productive biology for *R. brevipes*.

In analyses of the population variation of EM fungi using molecular tools, the size and number of genets has been used to measure their genetic diversity (see Redecker *et al.*, 2001). In most cases, there seems to have been a high density of EM genets sampled, with the exception of a few EM fungi that form large extensive genets including *Suillus*, *Xerocomus* and *Cortinarius* spp. (see Redecker *et al.*, 2001). The power of resolving genets is likely to be dependent on the frequency of alleles within populations and the number of loci analyzed. For example, in *Cantharellus formosus*, the co-occurrence of genets across plots separated by large distances suggests that the frequency of microsatellite alleles was insufficient for resolving novel genets in aggregates of basidiocarps (Dunham *et al.*, 2003). In these analyses, the expectation of encountering a genet of *R. brevipes* by chance was quite low because the allelic diversity was high across several loci; however, the saturation between sample size and allelic diversity was only reached in one population during a single fruiting season.

Perhaps it is not too surprising that no genetic structure was detected in the present study given the uniformity of the ecosystem studied and the relatively small spatial scale across which it was sampled. Moreover, it is also not too surprising that geographic structuring, and possibly cryptic speciation, was evident in a previous study based on a large-scale geographic sampling (Bergemann & Miller, 2002). The question that remains to be determined for populations of *R. brevipes* is how large are the genetic neighborhoods of this 'species' and or what is the distance between potentially isolated lineages or cryptic species? Populations of *R. brevipes* from western North America would be ideal for studying this, given that this region holds the world's greatest extent of mesic, temperate coniferous forests (Brunsfeld *et al.*, 2001) where *R. brevipes* is likely to occur. Furthermore, California has more recognized subspecies of animals and plants than any other region in North America of comparable size (Myers, 1990; Myers *et al.*, 1999). In western North America, disjunct populations between the Pacific coastal and the interior Rocky Mountain regions have been discovered in at least 60 plant, animal and fungal species (Brunsfeld *et al.*, 2001), and the genetic spatial structuring of taxonomic groups may coincide with the age of California's mountain ranges and aridification in the regions (Calsbeek *et al.*, 2003). A reasonable and testable hypothesis is that phylogeographic patterns of genetic structuring of *R. brevipes* occur in the mountains that surround the Great Central Valley of California and the coastal and interior ranges of the Pacific Northwest could be the focus of future research.

Acknowledgments

We would like to Rodrigo Olarte for assistance with the microsatellite amplifications and cloning, Nick Kordesch and Matt Smith for assistance with collecting and mapping, and Seth Kauppinenii for technical assistance. Funding was provided by the Betty & Gordon Moore Foundation, National Science Foundation DEB-0315607, DEB-9981711, DEB-9981548, USDA CREES 2003-01542 and EPSCoR 0047681.

References

Allen MF, Hipps LE. 1984. Comparative microclimates of some mycorrhizal fungi: requirements for long-range dispersal of spores. *Mycological Society of America Newsletter* **35**: 17.

Bergemann SE. 2002. *Population structure of Russula brevipes across stands, hosts and geographic regions*. PhD Dissertation, Laramie, WY: University of Wyoming.

Bergemann SE, Miller SL. 2002. Size, distribution, and persistence of genets in local populations of the late-stage ectomycorrhizal basidiomycete, *Russula brevipes*. *New Phytologist* **156**: 313–230.

Bergemann SE, Miller SL, Garbelotto M. 2005. Microsatellite loci from *Russula brevipes*, a common ectomycorrhizal associate of several tree species in North America. *Molecular Ecology Notes* **5**: 472–474.

Bonello P, Bruns TD, Gardes M. 1998. Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*. *New Phytologist* **138**: 533–542.

Brunsfeld SJ, Sullivan J, Soltis DE, Soltis PJ. 2001. Comparative phylogeography of North-western North America: a synthesis. In: Silvertown, J, Antonovics, J, eds. *Integrating Ecological and Evolutionary Process in a Spatial Context*. Oxford UK: Blackwell Science, 319–339.

Calsbeek R, Thompson JN, Richardson J. 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: the California Floristic Province. *Molecular Ecology* **12**: 1021–1029.

Chapela IH, Garbelotto M. 2004. Phylogeography and evolution in matsutake and close allies inferred by analyses of ITS sequences and AFLPs. *Mycologia* **96**: 730–741.

Dahlberg A. 2001. Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist* **150**: 555–562.

Dunham SM, Kretzer A, Pfrender ME. 2003. Characterization of Pacific golden chanterelle (*Cantharellus formosus*) genet size using co-dominant microsatellite markers. *Molecular Ecology* **12**: 1607–1618.

Fiore-Donno A-M, Martin F. 2001. Populations of ectomycorrhizal *Laccaria amethystina* and *Xerocomus* spp. show contrasting colonization patterns in a mixed forest. *New Phytologist* **152**: 533–542.

Gherbi H, Debaud J-C, Effose A, Marmeisse R. 2004. Below-ground distribution and persistence of an ectomycorrhizal fungus. *New Phytologist* **161**: 539–547.

Gherbi H, Delaruelle C, Selosse M-A, Martin F. 1999. High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150-year-old beech forest. *Molecular Ecology* **8**: 2003–2013.

Gregory PH. 1945. The dispersion of air-borne spores. *Transactions of the British Mycological Society* **28**: 1–55.

Griffin J. 1977. Oak woodlands. In: Barbour, MG, Malor, J, eds. *Terrestrial Vegetation of California*. New York, USA: John Wiley & Sons, 383–415.

Gryta H, Debaud J-C, Effosse A, Gay G, Marmeisse R. 1997. Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology* **6**: 353–364.

Gryta H, Debaud J-C, Marmeisse R. 2000. Population dynamics of the symbiotic mushroom *Hebeloma cylindrosporum*: mycelial persistence and inbreeding. *Heredity* **84**: 294–302.

Johnson CN. 1996. Interactions between mammals and ectomycorrhizal fungi. *Trends in Ecology and Evolution* **11**: 503–507.

Johnson PS, Shifley SR, Rogers R. 2002. *The Ecology and Silviculture of Oaks*. New York, NY: CABI Publishing.

Kretzer AM, Dunham S, Molina R, Spatafora JW. 2005. Patterns of vegetative growth and gene flow in *Rhizopogon vinicolor* and *R. vesiculosus* (Boletales, Basidiomycota). *Molecular Ecology* **14**: 2259–2268.

Kretzer AM, Molina R, Spatafora JW. 2000. Microsatellite markers for the ectomycorrhizal basidiomycete *Rhizopogon vinicolor*. *Molecular Ecology* **9**: 1190–1191.

Marshall TC, Slate J, Kruuk L, Pemberton JM. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639–655.

McDonald BA, Pettway RE, Chen RS, Boeger JM, Martinez JP. 1995. The population genetics of *Septoria tritici* (teleomorph *Mycosphaerella graminicola*). *Canadian Journal of Botany* **73**: S292–S301.

von Melzer C, Rothe GM. 2000. Genetic variability of two populations of the ectomycorrhizal fungus *Xerocomus chrysenteron* associated with European beech (*Fagus sylvatica* L.). *Forest Genetics* **7**: 85–96.

Molina R, Pilz D, Dunham S, Dreisbach T, O'Dell T, Castellano M. 2001. Conservation and management of forest fungi in the Pacific Northwestern United States: an integrated ecosystem approach. In: Moore, D, Nauta, MM, Evans, SE, Rotheroe, M, eds. *Fungal Conservation – Issues and Solutions*. Cambridge, UK: Cambridge University Press, 19–63.

Myers N. 1990. The biodiversity challenge: expanded hotspots analysis. *Environmentalist* **10**: 243–256.

Myers N, Mittermeier RA, Mitermeier CG, de Foseca GAB, Kents J. 1999. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535–538.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248–249.

Redecker D, Szaro TM, Bowman RJ, Bruns TD. 2001. Small genets of *Lactarius xanthogalactus*, *Russula cremoricolor*, and *Amanita francheti* in late-stage ectomycorrhizal successions. *Molecular Ecology* **10**: 1025–1034.

Rogers SO, Rogers MAM. 1999. Gene Flow in Fungi. In: Worrall, J, ed. *Structure and Dynamics of Fungal Populations*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 97–121.

Rousset F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**: 1219–1228.

Rousset F. 2000. Genetic differentiation between individuals. *Journal of Evolutionary Biology* **13**: 58–62.

Schneider S, Roeslli D, Excoffier L. 2000. *Arlequin. A Software for Population Genetics Data Analysis*. Geneva: University of Geneva, Genetics and Biometry Laboratory.

Selosse MA. 2003. Founder effect in a young *Leccinum duriusculum* (Schultzer) Singer population. *Mycorrhiza* **13**: 143–149.

Selosse MA, Martin F, Le Tacon F. 2001. Intraspecific variation in fruiting phenology in an ectomycorrhizal *Laccaria* population under Douglas fir. *Mycological Research* **105**: 524–531.

Smith SE, Read DJ. 1997. *Mycorrhizal Symbiosis*, 2nd edn. New York, USA: Academic Press.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.

Zhou Z, Miwa M, Hogetsu T. 2001a. Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal *Suillus grevillei* populations. *New Phytologist* **149**: 339–348.

Zhou Z, Miwa M, Matsuda Y, Hogetsu K. 2001b. Spatial distribution of the subterranean mycelia and ectomycorrhiza of *Suillus grevillei* genets. *Journal of Plant Research* **114**: 179–185.

