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Spatial analysis of within-population microsatellite variability reveals restricted gene flow in the Pacific golden chanterelle (*Cantharellus formosus*)

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Abstract: We examined the within-population genetic structure of the Pacific golden chanterelle (*Cantharellus formosus*) in a 50 y old forest stand dominated by Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) with spatial autocorrelation analysis. We tested the null hypothesis that multilocus genotypes possessed by chanterelle genets were randomly distributed within the study area. Fruit bodies from 203 *C. formosus* genets were collected from a 50 ha study plot. One hundred six unique multilocus genotypes were identified after scoring these collections at five microsatellite loci. Statistically significant positive spatial autocorrelation was detected indicating the presence of fine-scale genetic structure within the area. Repeated autocorrelation analyses with varied minimum distance classes (50–500 m) detected positive spatial genetic structure up to 400 m. Therefore nonrandom evolutionary processes (e.g., isolation by distance) can cause fine-scale genetic structure in *C. formosus*. The implications of this research for future broad-scale population studies of this species are that population samples should be separated by at least 400 m to be considered statistically independent. Sampling designs that account for fine-scale genetic structure will better characterize heterogeneity distributed across the landscape by avoiding the effects of pseudo replication.

Key words: correlogram, Ectomycorrhizal, fungi, genetic structure, spatial autocorrelation

INTRODUCTION

Concern for the population viability of many ectomycorrhizal (EM) fungi has drawn attention to the need for considering their conservation in Pacific Northwest forest management plans (Molina et al 2001). This task requires a basic understanding of population dynamics that can be advanced by population genetic research. Many genetic studies of EM fungal populations have characterized the spatial distribution of genets and collectively demonstrated differences in age structure, clonality and genet recruitment patterns among EM species (Dahlberg 2001, Guidot et al 2002). These studies have yielded insights into the relative roles of basidiospore dispersal and vegetative growth in EM fungal life histories. Several studies have documented small genets thought to have short persistence times (e.g. Gryta et al 1997, 2000; Gherbi et al 1999; Fiore-Donno and Martin 2001; Redecker et al 2001; Guidot et al 2002, 2003; Huai et al 2003; Sawyer et al 2003; Kretzer et al 2004, 2005) indicating that genet establishment from basidiospore germination likely is important for the maintenance of genetic diversity in EM fungal populations. Building on this knowledge by better characterizing the dispersal ability of EM fungi will increase our ability to integrate fungal population biology into forest management.

Evidence for long-distance dispersal among fungal populations comes from detection of abundant spores in the air, even upper strata of the atmosphere (Ingold 1971). In contrast direct measurements of fungal spore settlement have demonstrated steep gradients of reduced spore deposition with increasing distance (Wolfenbarger 1946, Gregory 1973, Lacey 1996, Morkkynen et al 1997), providing evidence for limited dispersal capabilities (<100 m). Results from these fine-scale spore settlement studies are mirrored by estimates of landscape-scale spore dispersal distances in the saprotrophic basidiomycete *Schizophyllum commune*. James and Vilgalys (2001) determined that trapped spores likely originated from local populations and proposed that genet establishment resulting from long-distance spore dispersal was rare despite the observation that spores of *S. commune* were common in the air. This hypothesis is supported in studies of EM fungi that have documented localized clustering of genotypes within populations of *Suillus grevillei* (Zhou et al. 2001), *Russula vinosa* (Liang et al. 2004) and

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Russula brevipes (Bergemann and Miller 2002) although the latter could be interpreted as interspecific differences because the populations shared almost no alleles.

In reality spore dispersal rates and distances measure only the potential for gene flow among populations. For EM fungi genet establishment also depends on basidiospore germination, growth of monokaryotic mycelia, dikaryon formation and successful colonization of host plant roots. Low EM basidiospore germination rates (<1%) have been observed in *Cantharellus* (Fries 1979) and other EM fungal species (Bonello et al 1998, Chamber and Cairney 1999), thus the large number of spores typically generated by EM fruit bodies may not reflect dikaryon formation and host root colonization rates (Dahlberg and Stenlid 1995).

If genet establishment resulting from long-distance dispersal (gene flow) is restricted in EM fungal populations, the probability of related individuals mating will increase as the distances between individuals decrease (isolation by distance, Wright 1969). Theoretical and empirical studies have shown that isolation by distance results in striking patterns of genetic structure within populations (Epperson 1993, Epperson et al 1999, Ueno et al 2000). Given the evidence for limited spore dispersal, it is reasonable to expect that fine-scale genetic structure may exist within EM fungal populations.

Patterns of genetic isolation often are estimated with the variance in allele frequencies within and among sampling units (Wright 1951, Slatkin and Barton 1989). Precision of these statistics requires that sampling units do not encompass multiple random breeding units (e.g. genetic neighborhoods, cryptic species; Magnussen 1993; Ruckelshaus 1998; Dunham et al 2003a). Wright (1969) showed analytically that as more neighborhoods are included within sampling units, the genetic variance among sampling units relative to the total variance declines and the power to detect spatial genetic structure is lost. This problem is important to consider in the study of EM fungal populations because the distributions of most species are not well documented (Dreisbach et al 2002). Such sampling pitfalls can be circumvented by using spatial autocorrelation analysis to characterize genetic structure because it directly analyzes genotypes possessed by individuals and no information is lost by pooling samples into arbitrary groups for subsequent comparisons of allele frequencies (Heywood 1991, Epperson 1993, Sork et al 1999). Because of this spatial autocorrelation analyses have increased power for detecting genetic differentiation over a range of spatial scales and can be used to define optimum strategies for conserving genetic variability within species (Sokal and Oden 1978, Sokal and Wartenberg 1983, Epperson 1993, Epperson and Li 1996, Epperson 1997, Smouse and Peakall 1999, Diniz-Filho and Telles 2002, Peakall et al 2003, Double et al 2005).

A practical approach to expanding our understanding of EM fungal population dynamics involves research on broadly distributed species that fruit reliably. Research on such species will impart the statistical power sufficient to detect evolutionarily important pattern-process relations (Dizon et al 1995). Pacific golden chanterelles (*Cantharellus formosus*) are commercially harvested basidiomycetes that form EM associations with many species of economically important host trees (Redhead et al 1997, Pilz and Molina 2002, Dunham et al 2003a). Individual fruit bodies of *C. formosus* can persist on the landscape, continuously producing basidiospores for up to 90 d (Largent and Sime 1995). Assuming wind is the primary mechanism of spore dispersal for *C. formosus* this species represents a distinct life history strategy for spore dispersal and provides a basis for comparison for spatial genetic analysis of other EM species. In this study we use microsatellite markers (Dunham et al 2003b) to characterize the fine-scale genetic structure in a *C. formosus* population. We employ quantitative criteria for assigning individual fruit-body collections to genets and assess the contribution of genet resampling to fine-scale genetic structure. We also estimate the contribution of isolation by distance to fine-scale genetic structure by analyzing genotypes of individual genets separately. We evaluated the alternative hypotheses of panmixia and isolation by distance by examining the relatedness of 203 genets separated by distances of 5–700 m.

METHODS

Study area.—Chanterelle fruit bodies were taken from a 50 ha (1 km × 0.5 km) plot on the southeastern border of the HJ Andrews Experimental Forest (44.2°N, 122.2°W) in Oregon's central Cascade Range. The plot is dominated by Douglas-fir (*Pseudotsuga menziesii*) and western hemlock (*Tsuga heterophylla*) naturally regenerated from clear-cut harvesting that occurred ~50 y ago. Understory vascular plants include *Polystichum munitum* in wet sites, *Berberis nervosa* in mesic sites and *Gautheria shallon* in drier sites. For several years before and during our research this area was closed to commercial and recreational harvest of fungi.

Field sampling.—During fall 1998, a sampling network that contained 12.2 km of transect length (20 m minimum distance between any two transects) was established and relative transect locations verified with a global positioning system (FIG. 1). The spatial coordinates of chanterelle fruit bodies within 2 m of

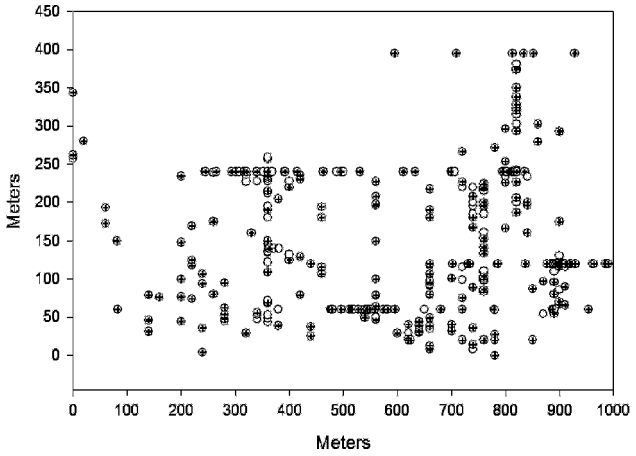

FIG. 1. Distribution of *C. formosus* collections used in all spatial analyses. Open circles represent 338 individual fruit body locations. Crosses represent the locations of the 203 individual genets identified with microsatellite loci. Fruit bodies with identical multilocus genotypes were considered to represent separate genets when separated by 20 m (expected multilocus genotype frequency ≥ 0.01) or 50 m (expected multilocus genotypes frequency < 0.01). Each unique genet is represented by a single fruit body (cross location). Gaps in the distribution represent areas that contained survey transects where chanterelles were not found.

any transect were recorded to the nearest 0.1 m. At several points during the field season mapped chanterelle locations were cross checked with a GPS. Mapping errors within transects and across neighboring transects were low (<0.5 m) but ranged up to 5 m across more distant transects. In 1997 Dunham et al (2003b) found that 72% of *C. formosus* genets characterized on several plots within the study area were less than 4 m across and all genets were under 15 m maximum width. To maximize the number of discrete genets sampled we maintained a minimum distance of 5 m between any two fruit bodies collected. The entire transect network was sampled between 1 Oct and 20 Nov 1998. During this time 4.4 km of transect length (36%) dispersed evenly throughout the study area was resampled to include data from individuals with delayed fruiting phenology (Selosse et al 2001).

Molecular data collection.—*C. formosus* is difficult to differentiate from a less common golden chanterelle that occurs in the study area (*C. cascadensis*, Dunham et al 2003a). To ensure that spatial analyses were carried out only on *C. formosus* we analyzed all fruit bodies with restriction fragment length polymorphism analysis of the internal transcribed spacer region of the nuclear ribosomal repeat (ITS-RFLP, Gardes and Bruns 1996; Horton and Bruns 2001) following methods described in Dunham et al (2003b). The ITS region was amplified with the fungal specific primer ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al 1990). Unpurified PCR products were digested separately with two restriction enzymes (*Hinf*I and *Hae*III) known to differentiate the two chanterelle species of interest.

All samples were scored at two tri- and two tetranucleotide microsatellite loci developed for *C. formosus* (Dunham et al 2003b). The samples also were screened at a 5th trinucleotide locus found to have scoring problems associated with null alleles. Information from this locus was used to improve genet resolution but was not included in subsequent analyses. PCR reactions were carried out in 20 μL volumes and contained 1 μL genomic DNA, 1× assay buffer A (Fisher Scientific), 200 μM dNTPs each, 0.25 μM of each primer, 1 U Taq DNA polymerase (Fisher Scientific). The PCR profile consisted of initial denaturation at 95 C for 3 min followed by 35 cycles (95 C, 45 s; 55 C, 60 s; 72 C, 60 s). After the 35th cycle samples were subjected to a final 72 C extension of 60 min. PCR products were analyzed on an ABI 377 automated sequencer with the GS500 Tamra internal size standard. Band sizes were determined with GENESCAN software (PE Applied Biosystems).

Genet assignment.—Dunham et al (2003b) found that the microsatellite loci used here do not fully resolve all *C. formosus* genets. To address this, we used both genetic and spatial information when assigning chanterelle fruit bodies to discrete genets. First we culled all collections so that any two fruit bodies possessing identical multilocus genotypes were separated by at least 20 m. This distance exceeds the maximum width (13 m) previously observed for chanterelle genets (Dunham et al 2003b) and accounts for mapping error introduced in this study. Remaining subsets of fruit bodies that possessed identical multilocus genotypes were culled further with expected multilocus genotype frequencies (Hardy-Weinberg assumptions) calculated from an independent sample set collected in the surrounding area (Dunham et al 2003b). Chanterelles are not known to produce mitotic (asexual) spores (Hutchinson 1989) so multilocus genotypes containing many low frequency alleles have low probabilities of being produced more than once via sexual reproduction. Previous chanterelle genet size research (Dunham et al 2003b) demonstrated that multilocus genotypes repeated over long distances (300 m to 11 km) always had relatively high expected frequencies (≥0.01%) indicating lack of marker resolution, while genotypes clustered at distances less than 15 m often had low expected frequencies (<0.01%) indicating clonal structures. Based on this we culled the remaining collections so that any two fruit bodies possessing identical multilocus genotypes were separated by at least 50 m if their genotype had an expected frequency < 0.01%. Similar methodology and values for expected multilocus genotype frequencies have been used to assign samples to genets in clonal plant species (Aspinwall and Christian 1992; Parks and Werth 1993; Reusch et al 1999a, 1999b).

Data analysis.—To examine the spatial distribution of genotypes from both individual microsatellite loci and combined multilocus genotypes we used the program GenAlEx V6 (Peakall and Smouse 2005, http://www.anu.edu.au/BoZo/GenAlEx/). This program employs multivariate analysis methods (Smouse and Peakall 1999) to calculate a correlation coefficient (*r*,

range −1 to +1) between genetic and geographic distances for all pairs of individuals within user specified distance classes. For example, *r* in the distance class 0–25 m would represent the correlation between genetic and geographic distances calculated between all pairs of individuals within 0–25 m of one another. We used the genotypic distance option to calculate linear genetic distances between all possible pairs of collections (Peakall and Smouse 2005) and selected distance classes that produced an even number of pairwise comparisons across all classes. To increase pattern resolution we selected the smallest distances classes that allowed sufficient sample size and statistical power (at least 500 pairwise comparisons) for calculating the *r* statistic. As a result distance class intervals used to explore isolation by distance were wider than those used to explore patterns due to vegetative growth.

For each distance class, statistical significance of *r* calculated from the data was determined by comparison to a randomized distribution of *r* values created via 1000 permutations that swapped genetic data across spatial locations. The resulting randomized distribution represents the expected behavior of the correlations statistic (given the data) under the null hypothesis that genotypes are distributed randomly across the study area. Randomized *r* values were sorted and the 25th and 975th values used to define upper and lower 95% confidence intervals (Smouse and Peakall 1999). Because we tested the hypothesis of isolation by distance our primary interest was the detection of positive autocorrelation within the shortest distance classes. We report accordingly one-tailed tests for positive spatial structure (Smouse and Peakall 1999, Peakall et al 2003) with an alpha of 0.05 used in hypothesis testing. Bootstrap errors for *r* within each distance class also were used as a more conservative significance test of the autocorrelation statistic (Peakall et al 2003). In combination *r* values were considered statistically significant when they exceed the 95% confidence interval around the null hypothesis of zero and their 95% bootstrap error interval did not contain zero. We estimated the extent of genetic autocorrelation in *C. formosus* by repeatedly testing for statistical significance of *r* in the shortest distance class in analyses that varied distance class size of 50–500 m by 50 m intervals (Peakall et al 2003, Double et al 2005).

RESULTS

Genet assignment.—Field sampling produced 366 chanterelle fruit bodies from the 50 ha plot, and 338 collections were confirmed as *C. formosus* by ITS-RFLP analysis. Microsatellite screening produced 106 unique multilocus genotypes from the 338 *C. formosus* collections. Culling samples so that at least 20 m (expected frequency ≥ 0.01) or 50 m (expected frequency < 0.01) separated fruit bodies possessing identical multilocus genotypes reduced the sample from 338 to 203 collections (FIG. 1). In the complete sample (338 collections) two multilocus genotypes were represented by 43 fruit bodies each. These multilocus genotypes were composed of the most frequent alleles for each locus and had expected frequencies of 0.08 and 0.02. The number of fruit bodies representing these multilocus genotypes was reduced to 13 and 20 respectively after distance criteria was applied.

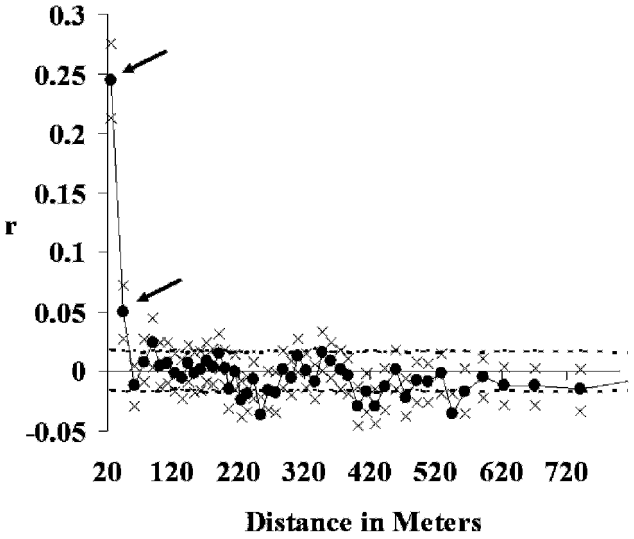

FIG. 2. A correlogram plotting the mean spatial autocorrelation determined from all *C. formosus* fruit bodies collected (n = 338). Each dot represents the mean correlation value per distance class bracketed in 95% bootstrap error bars (×). Dashed lines represent 95% confidence intervals determined with permutation tests. Arrows indicate statistically significant positive spatial autocorrelation values outside 95% confidence intervals with 95% bootstrap errors that do not overlap zero. The first x-axis intercept theoretically represents the extent of nonrandom genetic structure due to both clonal propagation and isolation by distance but is likely underestimated.

Spatial genetic structure due to clonal propagation.—The mean distance between fruit bodies in the total *C. formosus* sample (n = 338) was 9.4 m. Spatial autocorrelation analysis of genetic distances showed that multilocus genotypes were not randomly distributed (FIG. 2). Instead like genotypes clustered tightly at fine spatial scales with significant positive autocorrelation in the 0–25 m distance class ($r = 0.24$, $P < 0.001$). Statistically significant autocorrelation also was found in the 25–45 m distance class ($r = 0.05$, $P < 0.001$) indicating that either we have underestimated the extent of clonal propagation in *C. formosus*, or isolation by distance is also contributing to this fine-scale spatial structure. After dropping below the upper confidence interval the autocorrelation coefficient oscillates between positive and negative values. This pattern is indicative of strong fine-scale spatial structure

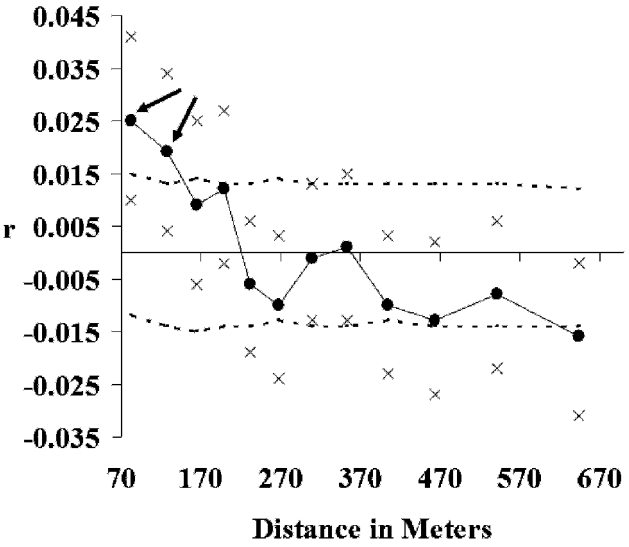

FIG. 3. A correlogram plotting the mean spatial autocorrelation values determined from all *C. formosus* genets identified (n = 203). Arrows indicate statistically significant positive spatial autocorrelation values outside 95% confidence intervals with 95% bootstrap errors that do not overlap zero.

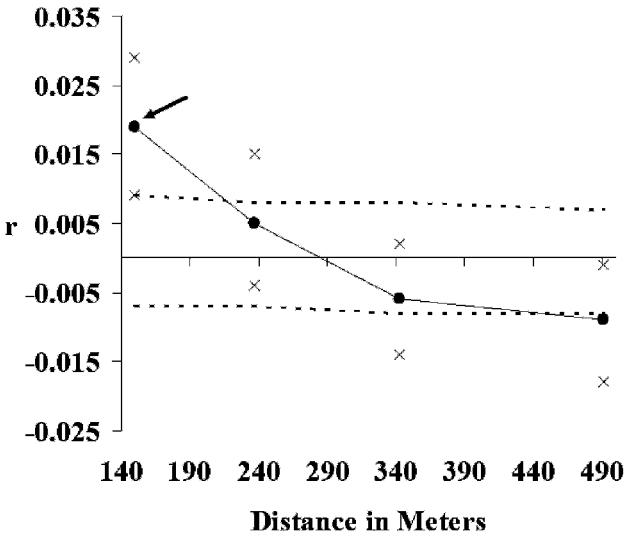

FIG. 4. A correlogram plotting the mean spatial autocorrelation values determined from all *C. formosus* genets sampled (n = 203) with the distance class size increased to better resolve the isolation by distance pattern detected in the analysis. Note the increase in the x-axis intercept with increasing distance class size compared to the previous figure.

created by discrete patches of similar multilocus genotypes repeated across the study area (Smouse and Peakall 1999, Diniz-Filho and Telles 2002). Separate analysis of each microsatellite locus (correlograms not shown) revealed that single locus genotypes are similarly structured across the study area.

Spatial genetic structure due to isolation by distance.—The contribution of clonal propagation was subtracted from the spatial autocorrelation analysis by reducing the sample to one randomly selected fruit body per genet (n = 203). The mean distance between *C. formosus* genets was 18.6 m. The correlation between geographic and multilocus genetic distance was positive and statistically significant in the shortest distance class (0–82 m, $r = 0.025$, $P = 0.002$) and in the 82–128 m distance class ($r = 0.019$, $P = 0.007$; FIG. 3). This analysis shows a clear trend toward increasingly negative autocorrelation values (statistically significant in the largest distance class) with increasing distance. This trend, indicative of isolation by distance (Diniz-Filho and Telles 2002, Peakall et al 2003) is more clear when distance class size is increased (FIG. 4).

Analyses of individual microsatellite loci showed that two of the four loci consistently mirrored the combined analysis while patterns for the other two loci fluctuated. The locus that always showed the strongest spatial pattern also had the highest observed heterozygosity (Cf145 = 0.69 vs. 0.44, 0.31 and 0.09 for Cf126, Cf339 and Cf642 respectively; Dunham et al 2003b). This variation in the direction and strength of signal in spatial autocorrelation analyses across loci is likely due to stochastic variability caused by random sampling of alleles during sexual reproduction (Smouse and Peakall 1999). As a result the multilocus analysis better demonstrates the general pattern of spatial affinity because it smoothes out the variance created by random genetic processes.

Spatial extent of genetic structure.—The radius of genetically homogenous patches resulting from isolation by distance as determined from the x-axis intercept (Epperson 1993, Epperson et al 1999) ranged from 220 m to 285 m (FIGS. 3, 4) and increased with increasing distance class size. Sequential tests for significantly positive spatial structure in the shortest distance class demonstrated that these x-axis intercepts underestimate the true extent of spatial structure in the study area (FIG. 5; Peakall et al 2003, Double et al 2005). As distance class size is increased from 50 to 500 m, the magnitude of r steadily declines. Bootstrap errors around r overlap zero when distance class size reaches 400 m. Further analysis of larger distance classes to the maximum extent possible for the data show r values converging on

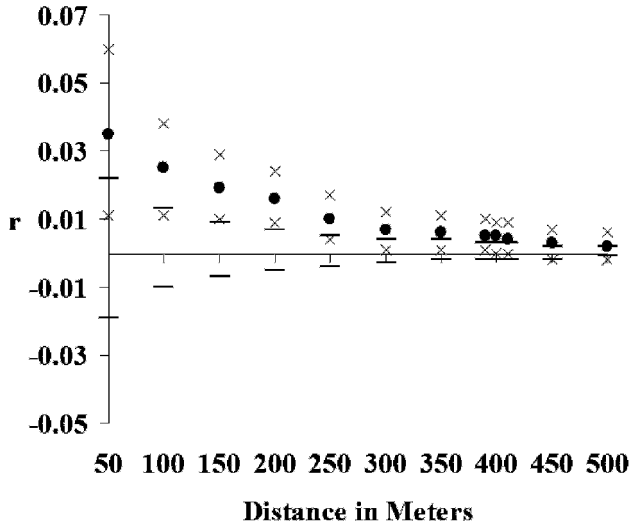

FIG. 5. A graph showing spatial autocorrelation values for the shortest distances calculated in sequential analyses increasing distance class size of 50–500 m. Dots represent r for the shortest distance class in each analysis. Dashes represent the 95% confidence interval relevant to the distance class in each analysis and × represents the 95% bootstrap errors estimated in each analysis. The lower bootstrap error intersects zero in the analysis where the shortest distance class is set at 400 m.

zero. Therefore the distance at which two genets are equally likely to be in the same patch or different patches within this 50 ha study area is approximately 400 m (Sokal and Oden 1978, Sokal and Wartenberg 1983, Epperson 1995).

DISCUSSION

The sign and direction of change in the spatial autocorrelation coefficient allows inference about evolutionary processes affecting a sample of individuals. Three common patterns detected include (i) random fluctuation of the autocorrelation coefficient around zero indicative of panmixia, (ii) a stabilizing profile with significant positive autocorrelation at short distances then random fluctuation of the autocorrelation coefficient around zero at longer distances indicative of patch structure (Sokal and Wartenberg 1983), and (iii) a long-distance cline with significant positive autocorrelation at short distances that decreases continuously with increasing geographic distance indicative of isolation by distance (Sokal et al 1997, Diniz-Filho and Telles 2002). While interpreting the empirical results of a correlogram pattern is fairly straight forward, drawing inferences about dispersal requires the ability to differentiate between all potential causes leading to spatial clustering of genotypes.

In fungi clonal (vegetative) growth confounds patterns of genetic structure and limits inferences about dispersal. The relationship we observed between genetic and geographic distance in the total *C. formosus* sample (known to contain multiple collections from discrete genets) indicates that clonal propagation is localized (FIG. 2). The correlogram for this analysis shows a stabilizing profile indicating the presence of many discrete homogeneous patches distributed throughout the study area. Independent estimates of genet width for *C. formosus* (Dunham et al 2003b) are much smaller than the extent of spatial dependence detected and agree with the large autocorrelation statistic found in 0–25 m distance class (FIG. 2). There are two possible explanations for the positive autocorrelation found in distance classes beyond 25 m. First fine scale inbreeding due to isolation by distance also exists within the population and has inflated the autocorrelation statistic beyond what is expected from simply re sampling genets. Second genets may be capable of short distance dispersal via vegetative growth of mycelia after fragmentation. The presence of genet fragments in proximity (but $>$ 20 m) to one another would increase the influence of vegetative growth on fine-scale population genetic structure. Given that our markers do not fully resolve all genets we cannot exclude this as a possible mechanism driving fine-scale genetic structure in *C. formosus*. To our knowledge this type of disjointed patch structure has not been detected in any other EM fungus for which genet size has been documented, therefore we find the second explanation unlikely.

After excluding replicate genet samples from the analysis we detected significant, positive autocorrelation of multilocus genotypes at short distances ($<$130 m) followed by steadily declining autocorrelation at longer distances (FIGS. 3, 4). The spatial analysis indicates that multilocus genotypes possessed by genets are distributed in a structured, isolation by distance pattern with a patch size of 400 m (FIG. 5). Significant genetic structure in the 0–82 m distance class may be due in part to repeated sampling of unusually large genets or fragmented genets (see above). This is unlikely given the highly significant genetic structure detected in the 82–128 m distance class (FIG. 3). For genet establishment via vegetative growth and fragmentation to occur at this scale mycelial growth rates would need to be fairly rapid, which contradicts laboratory observations of *Cantharellus* mycelial growth rates (Danell 1994).

Although statistically significant the strength of spatial genetic structure observed was low compared to other studies using the same analysis techniques (e.g. Smouse and Peakall 1999). It may be the case

that isolation by distance operates only weakly within *C. formosus* populations. Before drawing this conclusion we must consider the likelihood that our ability to detect spatial genetic patterns was compromised by low statistical power. Epperson et al (1999) demonstrated that maximum statistical power results from sampling sufficient numbers of individuals in at least 4–9 patches. Based on the estimated patch size and the total size of our plot, only 2–4 patches were sufficiently sampled. In addition Smouse and Peakall (1999) showed that the strength of spatial genetic signal increases with the level of polymorphism at each locus rather than with the total number of alleles sampled. Three of our four loci exhibited low heterozygote frequencies (0.09, 0.3 and 0.4) compounding the reduced power from inadequate sampling of patches.

Weak (although significant) spatial autocorrelation in the smallest distance classes indicates that close genets were not very similar. In other words the spatial pattern observed in overall genetic distances is better explained by long-distance differentiation than by short-distance similarity. Low positive autocorrelation at short distances coupled with slightly negative autocorrelation at longer distances can be interpreted two ways. The first explanation to consider is that despite the longevity of *C. formosus* fruit bodies (Largent and Sime 1995) gene flow is only partially sufficient to counteract fine-scale structure resulting from local inbreeding and genetic drift. Although EM fungi possess mating-type genes that typically function to reduce inbreeding (but see Bonello et al 1998), compatible basidiospores from the same genet can germinate and fuse to form a dikaryotic mycelium capable of fruit body formation. One the other hand we must consider that spore dispersal only represents potential gene flow and must be followed by successful germination, dikaryon formation and EM colonization for actual genet establishment to occur. Spores may be able to travel long distances but migrants may be selected against if they are not genetically compatible with EM host genotypes in the local area. Selection against spores from distant populations could create the patterns we observed if the monokaryons produced by long-distance dispersers are dependent on mating with monokaryons produced locally. Spores remaining locally would exhibit strong isolation by distance diluted by the introgression of alleles from outside the area.

Implications for future research on C. formosus.—Taken together the data presented above strongly support the hypothesis that dispersal is restricted in *C. formosus*. Regardless of the evolutionary mechanism driving the observed spatial genetic structure, our results have important implications for the design of population genetic studies at larger spatial scales and can be used to design research on various ecological patterns and processes at spatial scales relevant to management. The scale of positive genetic structure defines geographic distance between genetically independent samples (Diniz-Filho and Telles 2002). Collecting samples separated by this minimum distance will better characterize the genetically influenced heterogeneity distributed across the landscape by avoiding pseudo replication. For *C. formosus* these inferences currently are limited to the single site that we sampled in 50 y old second-growth Douglas-fir. Our research needs to be replicated under an array of environmental conditions before these conclusions could be considered widely applicable.

Implications for other EM species.—Population structure results from complex interactions between genetic, demographic and environmental factors. Characterizing genetic structure at multiple spatial scales provides a way to examine the processes that could affect the evolutionary potential and viability of a population (Dunham et al 1999). Studies on terrestrial plants have shown that dispersal capability and reproductive biology are correlated with genetic diversity, population structure and gene flow (Loveless and Hamrick 1984). Several studies of spore settlement have shown that most spores do not disperse far from the source (Wolfenbarger 1946, Gregory 1973, Lacey 1996, Morkkynen et al 1997). James and Vilgalys (2001) demonstrated that even though spores of *S. commune* were abundant in the air trapped spores showed population subdivision indicating lack of long-distance dispersal. In this study we have demonstrated that multilocus genotypes of *C. formosus* are structured at fine spatial scales in patterns consistent with isolation by distance despite the fact that fruit bodies persist on the landscape for long periods continually producing basidiospores (Largent and Sime 1995) albeit at unknown rates. Ectomycorrhizal fungi show substantial life history variability controlled both by genetic and environmental factors and offer numerous opportunities to investigate the relationship between life history strategy and dispersal capacity (Dahlberg 2001, Guidot et al 2002). General characterizations of dispersal capabilities for EM fungi will benefit a number of current research efforts aimed at understanding EM population biology and ecology by improving both predictions about establishment probability

for various species and knowledge of the scale at which sample independence occurs across landscapes (Wiens 1989).

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