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Population Biology

Randomly amplified polymorphic DNA markers are superior to somatic incompatibility tests for discriminating genotypes in natural populations of the ectomycorrhizal fungus *Suillus granulatus*

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ABSTRACT **Assessing genetic variation within populations and genetic exchange between populations requires an understanding of the distribution and abundance of individual genotypes within the population. Previous workers have used somatic incompatibility testing to distinguish clones or individuals in natural populations of ectomycorrhizal fungi. However, somatic incompatibility tests performed with isolates of *Suillus granulatus* from a natural population revealed a lack of transitiveness, which brought into question the validity of this method. Subsequent studies of genetic identity of these isolates, using randomly amplified polymorphic DNA (RAPD) markers, conclusively showed that somatically compatible isolates are not necessarily genetically identical. RAPD marker analysis is more reliable and provides higher resolution of genotype distribution in natural populations than does somatic incompatibility testing. This is of particular importance in populations of organisms such as ectomycorrhizal fungi in which the mating systems are incompletely known.**

To assess genetic variation within populations and genetic exchange between populations, the distribution and abundance of individual genotypes must be known. Determination of individual genotypes in ectomycorrhizal Basidiomycete populations is not trivial, however, because the dikaryotic vegetative thalli of these fungi grow below ground in association with tree roots. An individual thallus may produce one or more dikaryotic fruiting bodies (mushrooms), which indicate the local occurrence of a species but provide no information regarding the abundance of distinct genotypes (individuals) in the population.

Researchers working with wood-decomposing and tree-pathogenic Basidiomycetes (1) have used somatic incompatibility testing for delineating different genotypes, which are frequently called clones or individuals. Somatic compatibility is assessed via confrontations of isolates from different fruiting bodies on nutrient media. If isolates produce a zone of somatic rejection between them (growth inhibition or compatible with B and C, then B and C must be compatible as well.

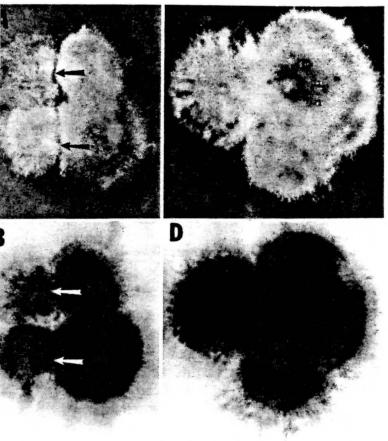
Attempts to determine the genetic control of somatic incompatibility in the Basidiomycetes have not been successful. Rayner and Todd (2) thus concluded that somatic incompatibility is polygenically controlled.

Somatic incompatibility testing has recently been applied to the description of ectomycorrhizal fungal populations. Fries (11) concluded that more than one clone of *Suillus luteus* (L. ex Fr.) S. F. Gray was associated with a single host tree. Dahlberg and Stenlid (12) used somatic incompatibility to investigate the "spreading strategy and clonal size" of *Suillus bovinus* (L. ex Fr.) Kuntze populations in pine forests with different stand histories. Finally, Sen (13) showed that somatic incompatibility reactions could be correlated with isozyme patterns for delineating individuals of *S. bovinus* and *Suillus variegatus* (Swartz ex Fr.) Kuntze.

We report here somatic incompatibility tests performed with isolates of *Suillus granulatus* (L. ex Fr.) Kuntze from a natural population, which caused us to question the assumption that somatically compatible isolates of this ectomycorrhizal fungus are from a single genetic individual (i.e., have the same genotype). We subsequently used randomly amplified polymorphic DNA (RAPD) markers to determine whether somatically compatible *S. granulatus* isolates were genetically identical. RAPD marker analysis (14, 15) has recently been used in the delineation of distinct genotypes in closely related individuals in a wide range of organisms, including fungi (16–21). In contrast to somatic incompatibility testing, isolates are determined to be from the same individual only if they have identical patterns for the presence or absence of markers at the loci examined.

METHODS

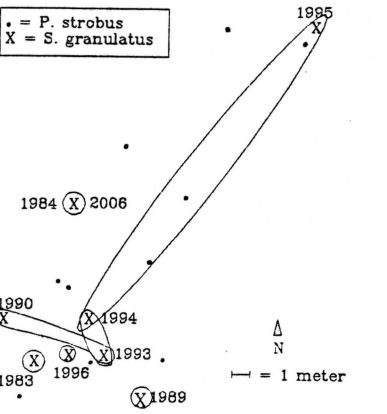
Study Site and Fieldwork. A study site (300 m^2) was



1. (*A* and *B*) Somatic incompatibility of isolates VT1993 nd VT1996 (right), as indicated by a barrage zone (arrows) n the isolates. (*A*) View of top with above lighting. (*B*) View om with above lighting. (*C* and *D*) Somatic compatibility of VT1994 (left) and VT1995 (right), as indicated by confluent between the isolates. (*C*) View of top with above lighting. (*D*) f bottom with above lighting.

5 prevented their delineation as discrete individuals 2). (For example, somatic compatibility between 0 and VT1993 suggested that they were from the same ual, as did somatic compatibility between VT1993 and 4. However, VT1994 and VT1990 were not compatible e thus from different individuals.)

D Marker Analysis. Five primers yielded a total of 47 markers (Table 1). (Fig. 3 illustrates the RAPD s identified with three of the primers.) A phenetics m, NTSYS (23), was used to quantify similarities be- the nine isolates. A similarity matrix, obtained via the



. Results of somatic incompatibility tests for *S. granulatus* from the Poverty Creek site. Encircled isolates were somat- mpatible. The ectomycorrhizal tree associates, *P. strobus*, indicated.

Table 1. Data matrix of the RAPD markers identified from population isolates by primers OPA-7 (A7), OPB-18 (B18), OPB-10 (B10), OPA-13 (A13), and OPA-16 (A16).

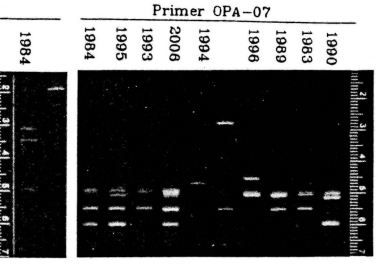
	Population isolate								
Marker	1984	1995	1993	2006	1994	1996	1989	1983	1990
A13:300	0	0	0	0	1	0	0	9	0
A13:350	0	0	0	0	0	1	0	9	0
A13:380	0	0	1	0	1	1	1	9	1
A13:400	0	0	1	0	0	0	0	9	0
A13:430	0	0	1	0	1	0	1	9	0
A13:470	0	0	1	0	0	1	1	9	0
A13:500	1	1	1	1	1	1	1	9	1
A13:630	0	0	0	0	0	0	1	9	0
A13:650	0	0	1	0	0	1	1	9	1
A13:900	1	1	1	1	1	1	1	9	1
A13:1050	1	1	0	0	0	0	1	9	1
A13:1100	1	0	1	1	0	1	1	9	1
A16:420	0	0	0	0	1	0	0	0	0
A16:470	0	0	0	0	0	0	1	0	0
A16:700	1	1	1	1	1	1	1	1	1
A16:850	1	1	1	1	0	0	1	1	1
A16:1050	1	1	1	1	0	0	1	1	1
A16:1400	1	1	0	1	0	1	1	1	0
A16:1500	1	1	0	1	0	0	0	0	0
B18:280	0	0	0	0	1	0	0	0	0
B18:330	0	0	0	0	1	0	0	0	0
B18:350	0	0	0	0	1	0	0	0	0
B18:390	0	0	0	0	1	0	0	0	0
B18:450	1	0	0	1	1	0	0	0	0
B18:480	1	1	1	1	0	1	1	1	1
B18:500	0	1	0	0	1	1	1	0	0
B18:530	0	0	0	0	1	0	0	0	0
B18:580	0	1	0	0	0	0	0	0	0
B18:630	1	0	1	1	1	1	1	1	1
B18:700	0	0	0	0	1	0	0	0	0
B18:760	1	1	1	1	1	0	1	0	0
B18:820	1	1	0	1	0	0	1	0	1
B18:950	0	0	0	0	1	0	0	1	0
B18:1100	1	1	0	1	0	1	0	0	1
B18:1200	1	1	1	1	1	1	1	1	1
B18:1300	1	1	1	1	1	1	1	1	1
A7:500	1	1	0	1	0	0	0	0	1
A7:600	1	1	1	1	0	0	1	1	0
A7:680	1	1	0	1	0	0	0	0	1
A7:720	1	1	1	1	0	1	1	1	1
A7:800	0	0	0	0	1	0	0	0	0
A7:850	0	0	0	0	0	1	0	0	0
B10:520	0	0	0	0	1	0	0	0	0
B10:600	0	0	0	0	1	0	0	0	1
B10:700	1	1	1	1	0	1	1	1	1
B10:820	1	1	1	1	0	1	1	1	1
B10:1300	1	1	1	1	1	1	1	1	1

0, Marker not present; 1, marker present; 9, data missing. Marker numbers refer to the primer used and band mobility (in bp).

dice coefficient (equivalent to the band sharing coefficient), was used to construct a phenogram via the unweighted pair group method (23). The phenogram represented an excellent fit of the data, as determined by the cophenetic correlation coefficient (r = 0.97078).

RAPD marker analysis indicated that this population was composed of at least eight genotypes exhibiting various degrees of presumptive genetic relationship. VT1994 and VT1983 were the most genetically distinct genotypes in the population, having, respectively, only 39% and 56% similarity with other isolates from the population at the loci examined. The remaining isolates were at least 70% similar to one another and clustered around two regions of high genetic similarity: isolates VT1984, VT2006, and VT1995 (93% similar); and isolates VT1993 and VT1989 (85% similar). Isolates VT2006 and VT1984 were most similar, with only one difference at the 47 markers examined (98% similar).

Comparing the Two Methods. The data from the somatic incompatibility tests were used to construct a similarity matrix using the simple matching coefficient (23). A phenogram was constructed using the similarity matrix via the unweighted pair group method of NTSYS (23). To quantify the agreement between the somatic incompatibility tests and RAPD marker analysis, the product-moment correlation for the phenograms was computed by using the matrix comparison option of NTSYS (23). There was no correlation between the similarity matrices (Fig. 4) obtained from the two analyses (r = 0.01). It is obvious then that the two methods produced



from nine isolates from the Poverty Creek site. RAPD reaction
TP, and dCTP; 47 ng of random 10-mers (Operon Technologies,
were conducted in a Perkin–Elmer thermocycler with an initial
healing), 36°C for 1 min (polymerization), and 93°C for 1 min
se with 1% Synergel (Diversified Biotech) and 1× TAE buffer.

tic incompatibility tests with ectomycorrhizal fungi is
ssary. Somatic incompatibility tests are known to be
ctual for determining genetic identities of closely re-
individuals (2, 6, 7, 10, 16, 24, 25). Dikaryons synthe-
from monokaryotic spores from a single basidiocarp
composed dikaryons) may or may not be compatible with
other and the parent dikaryon. Levels of incompatibility
pparently species specific but were as high as 50% in the
othallic, tetrapolar Basidiomycetes examined (inbreed-
otential of 25%). In most of these studies, populations
composed of numerous different individuals as deter-
d by high levels of somatic incompatibility between
es. However, based on the backcrosses conducted with
mposed and parent dikaryons, these authors cautioned
somatic incompatibility might erroneously indicate ge-
identity of closely related but genetically different
iduals if they had occurred in the natural populations.
ting systems in ectomycorrhizal fungi have not been
studied primarily because of difficulties encountered
spore germination. However, there are numerous rea-
to suspect that *Suillus* populations, if not all ectomy-
izal fungi, may be composed of closely related individ-
which would render somatic incompatibility testing
d for distinguishing different genotypes. To date, the
g systems of *S. granulatus* (Swedish isolates only), *S.*
s, and *S. bovinus* are known to be heterothallic and
ar (26, 27), resulting in an inbreeding potential of 50%.
dition, it is unknown but suspected that ectomycorrhizal
iomycete individuals are perennial, living for many
in association with the roots of the host plant. An old
ycorrhizal population may thus be composed of nu-
us sexual generations of individuals coexisting at one
exhibiting various levels of genetic relatedness.
llus species are also known to possess dikaryotic in
on to monokaryotic spores (28). Studies with *S. gran-*
s indicate that the dikaryotic spores are heterokaryotic
Theoretically, the resultant secondary homothallism
d contribute to the inbreeding potential of natural pop-
ns of *S. granulatus*, perhaps also accounting for the
f transitiveness observed in our study.
r data caution against the sole use of somatic incom-
lity testing for determining distributions of genotypes in
ations of other ectomycorrhizal Basidiomycetes. The
tial longevity of ectomycorrhizal populations, and mat-
stems that might result in high levels of inbreeding,
ves further investigation prior to assuming that ecto-
rrhizal populations are similar in genetic structure to
-decomposing and root-pathogenic Basidiomycetes.

Moreover, a compatible reaction provides no information regarding the genetic identity of individuals at any other loci; use of the terms clone or individual may be inappropriate for describing the outcome of somatic incompatibility studies without further investigations of the genetic identity of these fungi. Our study shows that RAPD marker analysis provides an effective, easy, and rapid means for examining the distribution of genotypes in natural fungal populations, in which mating systems are not well characterized.

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