Genetic diversity and population structure of the Italian fungi belonging to the taxa *Pleurotus eryngii* (DC.:Fr.) Quèl and *P. ferulae* (DC.:Fr.) Quèl

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A study using allozymes and PCR fingerprinting was conducted to estimate the genetic diversity of Italian populations of two economically important cultivated fungal taxa, *Pleurotus eryngii* and *P. ferulae*. Very little is known about the genetic diversity distribution pattern of these taxa. Heterozygote deficiency was observed at few loci; in fact the inbreeding coefficients were not high, which demonstrates that mechanisms restrain the inbreeding act at the local level. Estimates of genetic differentiation indicated a pattern of greater variation within, rather than between, populations.

These results were supported by AMOVA analysis, which attributed a low proportion of the total genetic variation to large geographical scale divergence, and indicated that most of the genetic diversity was because of differences within populations. This distribution pattern of genetic variation of *P. eryngii* and *P. ferulae* populations seems to be the result of high gene flow, by efficient basidiospore dispersal, and outcrossing mechanisms, which restrain inbreeding within populations.

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Introduction

Understanding the patterns of genetic diversity of species is fundamental not only for addressing questions concerning evolutionary processes and the development of conservation strategies, but also as a prerequisite for the efficient use of genetic resources in breeding programs. The interest in the genetic structure of natural populations has increased in the last few years owing to the necessity to broaden the knowledge of genetic variation in cultivated species. New approaches to the study of genetic variation from wild species to cultivated varieties, mediated by information on molecular markers, are promising avenues to exploit wild genetic resources. With fungi, breeding programs can be hindered by the lack of data on the extent of genetic variation in the natural populations. In fact, despite the economic importance of species in general or fungi in particular, little was known until recently about their natural history and evolutionary biology, for example, the evolutionary forces shaping natural populations and the available genetic variability.

The 11 species of the genus *Pleurotus* (Basidiomycota) are among the more conspicuous fungi causing wood decay in terrestrial ecosystems worldwide, and are widely collected and cultivated as edible fungi (Chang, 1993; Chang 1996). They exhibit bipolar heterothallism; single-spore cultures from individual fruit bodies are of

two kinds with several mating types in the population that efficiently promote outbreeding. The interaction of compatible monokaryons, derived from single basidiospores, results in a dikaryon (Elliot, 1994). There are few investigations on the population genetics of these species, and these only concern cultivated species, such as *P. ostreatus* (Jacq.: Fr.) P. Kumm.

The *P. eryngii* (DC.: Fr.) Quèl complex is distributed in the temperate Mediterranean region, in South Germany, Hungary, Slovakia and in Central Asia. Four varieties belong to this complex, two of which have recently been classified as separate species: *P. eryngii* and *P. ferulae* Authority (Urbanelli *et al*, 2002). The two taxa, morphologically similar in the juvenile phase, can be discriminated at maturity by the shape, color and diameter of the pileus and the size of the basidiospore (Hilber, 1982). They colonize the roots and the stems of some Apiaceae: *P. eryngii* that prevalently grows on the roots of *Eryngium campestre*, distributed throughout Western Europe, and *P. ferulae* grows mostly on the roots of *Ferula communis*, distributed throughout the Mediterranean region. These two host plants occur widely in Italy. Both mushroom taxa have been described as weak parasitic entities of these plants, although according to some authors they exhibit certain differences: *P. eryngii* is mainly saprophytic, while *P. ferulae* is mostly pathogenic (Cailleux *et al*, 1981; Urbanelli *et al*, 2002). Recent studies conducted on the genetic differentiation and the reproductive isolation of *P. eryngii* and *P. ferulae* populations have revealed that they are two distinct, genetically and reproductively isolated, entities (Urbanelli *et al*, 2002). The identification of molecular markers has allowed a clear discrimination of the two taxa, and the analysis of sympatric

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populations. The industrial production of these two taxa, under one name, *P. eryngii*, is well-developed in Italy and is currently under way in Europe. Hence, the need for a thorough knowledge of the genetic variation within and among populations of the two species.

In this paper, we report data on the use of informative markers systems to quantify the genetic diversity and understand the structure of natural Italian populations of *P. eryngii* and *P. ferulae*. We make inferences on the mating system and other factors affecting the observed levels and patterns of genetic variation.

Materials and methods

Studied samples and their geographic origin

We collected 123 wild fruitbodies of *P. eryngii*, near host *E. campestre* plants in six Italian localities and 261 wild fruitbodies of *P. ferulae* near *Ferula communis* in 13 Italian localities (Table 2). According to the sampling method, different fruitbodies might be derived from the same mycelium. To avoid the consequences of including the same individual several times, we collected each individual fungus from near different plants and at least 1 or 2 m apart.

The samples were morphologically identified by shape, color and diameter of pileus and size of the basidiospore.

Fruitbodies enzymatic extraction

Once in laboratory, each specimen was cut into small pieces and frozen in Eppendorf tubes at -80°C for later electrophoresis. Single pieces of fruitbodies were ground and dissolved in an extraction buffer (20 mM Tris-HCl at pH 7, 2 mM EDTA, 14 mM β -mercaptoethanol, 10 mM MgSO_4 , 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 0.1 M sorbitol) modified from Chalot *et al* (1989).

Allozyme gel electrophoresis

Homogenates were absorbed into 3×5 mm pieces of chromatography paper (Whatman 3MM) and placed on 10% starch gels. Standard horizontal electrophoresis was carried out at 7–8 V/cm for 4–6 h at 5°C . We analyzed up to 14 enzyme systems for each sample, encoded by 18 putative loci: α -Glycerophosphate dehydrogenase (α -Gpdh, EC 1.1.1.8), lactate dehydrogenase (*Ldh*, EC 1.1.1.28), malate dehydrogenase (*Mdh*-1, *Mdh*-2, EC 1.1.1.37), isocitrate dehydrogenase (*Idh*-1, *Idh*-2, EC 1.1.1.42), NADH Diaphorase (*Dia*, EC 1.6.2.2), superoxide dismutase (*Sod*-1, EC 1.15.1.1), aspartate aminotransferase (*Aat*-1, *Aat*-2, EC 2.6.1.1), phosphoglucosyltransferase (*Pgm*, EC 5.4.2.2), leucine aminopeptidase (*Lap*, EC 3.4.11), peptidase (Leu-Ala) (*Ap*-1, *Ap*-2, EC 3.4.11), peptidase (Leu-Leu) (*Pep*-2, *Pep*-3, EC 3.4.11), Mannose phosphate isomerase (*Mpi*, EC 3.4.11), glucose phosphate isomerase (*Gpi*, EC 5.3.1.9). These loci included $\approx 50\%$ of fast- and 50% of slow-evolving loci, *sensu* Koehn and Eanes (1978). The electrophoretic procedures are reported in detail in Urbanelli *et al* (2002).

Allozymes were enumerated according to their decreasing mobility (from the most anodal) relative to the most common allele (=100) in a reference population (Monte Romano, Latium).

PCR-fingerprinting gel electrophoresis

Total genomic DNA was extracted from 15 mg of mycelia according to Noel and Labarère (1987). The primer used for PCR amplification was the internal repetitive sequence of bacteriophage M13 DNA (5'-GAGGGTGGCGGTTCT-3') (Latouche *et al*, 1997). The band pattern that originated after 2 h (90 V) agarose gel (1.2%) electrophoresis was analyzed by the Gel-Compare 3.1 software (Applied Maths, Kortrijk, Belgium).

Allozyme data analysis

Allele frequencies were calculated for each population. Genotypes were computed for each individual in a population and used to estimate genetic parameters.

Genotype frequencies were tested for departures from Hardy-Weinberg equilibrium expectations with the exact probability test implemented by BIOSYS-1 (Swofford and Selander, 1981). The extent of genetic variation in each population was quantified by determining the number of alleles per locus (A), the percentage of polymorphic loci (P), 95% criterion, and the unbiased heterozygosity (H). The samples were of similar size and so the bias in the parameters of genetic variability did not affect the conclusions. The index of inbreeding, defined as $F_{IS} = 1 - (H_0/H_E)$, was calculated for each population over all polymorphic loci, where H_0 and H_E correspond to average observed and expected heterozygosities, respectively. The probability of outcrossing t was estimated using the equilibrium value of F_{IS} , which is defined as $(1-t)/(1+t)$ or $t = (1-F_{IS})/(1+F_{IS})$ (Hartl, 1987).

Gene diversity statistics were obtained using the method of Nei (1987). The total allelic diversity is represented by H_T , which is partitioned into the mean allelic diversity within population, H_S , and the allelic diversity among populations, D_{ST} . These quantities are related by the expression $H_T = H_S + D_{ST}$. The proportion of the total allelic diversity found among populations, G_{ST} , was calculated as the ratio D_{ST}/H_T .

The gene flow among populations, or genetically effective migration rate (Nm), was estimated using Wright's F_{ST} (Wright, 1951). The effective migration rate, estimated by the fixation index F_{ST} , is defined with the formula $Nm = (1/F_{ST}) - 1/4$ (Wright, 1969; Slatkin, 1987). This formula assumes the infinite-island model of population structure and gene flow; very few populations conform to this assumption, but it provides a useful approximation of the relative magnitude of gene flow.

PCR-fingerprinting data analysis

All individuals were scored for the presence or absence of fingerprinting fragments, and the data were entered into a binary data matrix as discrete variables (1 = presence; 0 = absence). Four populations of *P. eryngii* and four population of *P. ferulae* were studied (Table 4). The same specimens analyzed using isozymes were used for PCR-fingerprinting analysis. To generate similarity matrices, the data were analyzed using Excoffier *et al* (1992) algorithm, with the software RAPD analysis, ver. 1.02 (Armstrong *et al*, 1993).

The AMOVA analysis was performed on the fingerprinting haplotypes, and the Euclidean distances were calculated between all pairs of haplotypes (Excoffier *et al*, 1992; Huff *et al*, 1993). This analysis was used to split the total genetic variation into that occurring within populations

$[\Phi_{ST} = (\sigma_a^2 + \sigma_b^2) / \sigma^2]$, among populations within regions ($\Phi_{SC} = \sigma_a^2 / \sigma^2$) and between regions [$\Phi_{CT} = \sigma^2 / (\sigma_b^2 + \sigma_c^2)$]. The associated test for the homogeneity of molecular variance was carried out. All the variance components were tested statistically by nonparametric randomization tests using 2000 permutations. These analyses were performed with the WINAMOVA program provided by Excoffier (University of Geneva, Switzerland).

Results

Allozyme analysis

Genetic polymorphism: The 18 putative enzyme loci investigated allowed us to study the genetic variation of six populations of *P. eryngii* and 13 populations of *P. ferulae*. Polymorphism was observed at 13 enzyme loci (*Ldh*, *Mdh-1*, *Mdh-2*, *Idh-2*, *Dia*, *Aat-1*, *Aat-2*, *Pgm*, *Ap-2*, *Pep-2*, *Pep-3*, *Mpi* and *Gpi*). Among these, four (*Mdh-2*, *Dia*, *Pep-2* and *Pgm*) were diagnostic among taxa, allowing clear identification of *P. eryngii* and *P. ferulae* samples from the field (Table 1). The results of Hardy–

Table 1 Characteristic alleles at the four loci found to be diagnostic between *P. eryngii* and *P. ferulae*

Loci	Taxa	
	<i>Pleurotus eryngii</i>	<i>Pleurotus ferulae</i>
<i>Mdh-2</i>	114, 118	86, 94, 100, 116
<i>Dia</i>	98, 102, 106, 112,	94, 100
<i>Pep-2</i>	88, 92	100
<i>Pgm</i>	88, 90, 96, 98	92, 100, 104

Weinberg tests showed that genotype frequencies at most polymorphic loci did not depart significantly from the expected values. Significant deviations ($P < 0.05$), always associated with heterozygote deficiencies, were observed in the *P. eryngii* populations for the loci *Ldh* (population 1), *Mdh-2* (2), *Dia* (6) and *Mpi* (3–6), and in the *P. ferulae* populations for the loci *Ldh* (9 and 10), *Dia* (13 and 14), *Pgm* (19), *Mpi* (7, 11, 12, 14–16 and 18) and *Pgi* (18).

The genetic variability parameters were higher in *P. eryngii* than in *P. ferulae* samples (Table 2). The most variable *P. eryngii* population was Monte Romano ($A = 2.1$, $P_{95} = 0.72$ and $H_o = 0.240$); the least variable was Ceglie Messapico ($A = 1.4$, $P_{95} = 0.33$ and $H_o = 0.115$). Within *P. ferulae* the most and variable population was Castel del Monte ($A = 1.6$, $P_{95} = 0.44$ and $H_o = 0.165$); Macomer was the least variable ($A = 1.5$, $P_{95} = 0.39$ and $H_o = 0.085$).

The estimates of the inbreeding coefficient (F_{IS}), averaged over all polymorphic loci in each population, were positive and only in one case were they significant. The F_{IS} values were 0.235 in *P. eryngii* and 0.262 in *P. ferulae*, reflecting a low autogamy rate. The outcrossing rates, estimated from the F_{IS} index averaged over all polymorphic loci, were high, ranging from 0.485 to 0.914 (mean = 0.632) in *P. eryngii* and from 0.416 to 0.721 (mean = 0.592) in *P. ferulae* (Table 2).

Genetic diversity and gene flow: Averaged over all loci, the total genetic diversity H_T was 0.233 for *P. eryngii* and 0.172 for *P. ferulae* taxa (Table 3). The genetic diversity within populations was responsible for most of this diversity ($H_S = 0.212$ in *P. eryngii* and $H_S = 0.165$ in *P. ferulae*). In fact, the coefficients of genetic differentiation among populations, $G_{ST} = 0.100$ in *P. eryngii* and 0.045 in *P. ferulae*, showed that a large amount of genetic diversity lies within populations. Genetic differentiation between

Table 2 Values of genetic variability in Italian populations of *P. eryngii* and *P. ferulae*

Code	Populations	N	A	P_{95}	H_o	H_E	s.e.	F_{IS}	t
<i>Pleurotus eryngii</i>									
1	Monte Romano (Latium)	24	2.1	0.72	0.240	0.285	0.05	0.158	0.727
2	Castel del Monte (Apulia)	18	1.8	0.61	0.179	0.258	0.03	0.306	0.531
3	Ceglie Messapico (Apulia)	18	1.4	0.33	0.115	0.176	0.05	0.347	0.485
4	Nulvi (Sardinia)	18	1.6	0.33	0.141	0.181	0.07	0.221	0.638
5	Mamone (Sardinia)	20	1.6	0.39	0.123	0.185	0.05	0.335	0.498
6	Macomer (Sardinia)	25	2.1	0.31	0.170	0.178	0.06	0.045	0.914
	Mean	20.5	1.8	0.45	0.161	0.211	0.05	0.235	0.632
<i>Pleurotus ferulae</i>									
7	Monte Romano (Latium)	18	1.7	0.44	0.136	0.164	0.03	0.171	0.708
8	Tarquinia (Latium)	18	1.6	0.39	0.154	0.189	0.06	0.185	0.688
9	Castel del Monte (Apulia)	21	1.6	0.44	0.165	0.197	0.06	0.162	0.721
10	Poggiorsini (Apulia)	16	1.4	0.39	0.087	0.148	0.05	0.412	0.416
11	Gravina (Apulia)	22	1.3	0.31	0.087	0.110	0.05	0.209	0.654
12	Mamone (Sardinia)	20	1.8	0.50	0.123	0.198	0.04	0.379	0.450
13	Campeda (Sardinia)	20	1.6	0.50	0.146	0.192	0.04	0.240	0.613
14	Nuoro (Sardinia)	16	1.6	0.44	0.092	0.148	0.04	0.378	0.451
15	Macomer (Sardinia)	22	1.5	0.39	0.085	0.129	0.04	0.341	0.491
16	Borore (Sardinia)	24	1.5	0.33	0.115	0.153	0.05	0.248	0.603
17	Santu Lussurgiu (Sardinia)	18	1.4	0.39	0.088	0.113	0.04	0.221	0.638
18	Abbasanta (Sardinia)	22	1.6	0.31	0.105	0.144	0.05	0.271	0.574
19	Paulilatino (Sardinia)	24	1.6	0.39	0.135	0.165	0.05	0.182	0.692
	Mean	20.1	1.6	0.40	0.117	0.158	0.04	0.262	0.592

A, mean number of alleles per locus; P_{95} , proportion of polymorphic loci at 95% criteria; H_o , observed average genetic heterozygosity per locus; H_E , expected average genetic heterozygosity per locus; se, standard error; F_{IS} , inbreeding coefficient estimates; t, outbreeding coefficient estimates.

Table 3 Genetic diversity estimates for the Italian populations of *P. eryngii* and *P. ferulae*

Loci	<i>Pleurotus eryngii</i>				<i>Pleurotus ferulae</i>			
	H_T	H_S	D_{ST}	G'_{ST}	H_T	H_S	D_{ST}	G'_{ST}
<i>Ldh</i>	0.134	0.138	-0.004	-0.035	0.362	0.369	-0.007	-0.021
<i>Mdh-1</i>	0.020	0.020	0.000	0.002	0.057	0.055	0.002	0.036
<i>Mdh-2</i>	0.201	0.197	0.003	0.018	0.123	0.121	0.002	0.018
<i>Idh-2</i>	0.096	0.092	0.004	0.047	0.059	0.055	0.004	0.072
<i>Dia</i>	0.631	0.492	0.139	0.241	0.066	0.066	0.001	0.013
<i>Aat-1</i>	0.065	0.067	-0.003	-0.049	0.167	0.163	0.004	0.026
<i>Pgm</i>	0.391	0.374	0.017	0.048	0.325	0.301	0.024	0.080
<i>Ap-2</i>	0.066	0.062	0.003	0.058	0.105	0.100	0.005	0.046
<i>Pep-2</i>	0.064	0.066	-0.002	-0.028	—	—	—	—
<i>Pep-3</i>	0.058	0.059	-0.001	-0.015	0.012	0.012	0.000	0.015
<i>Mpi</i>	0.770	0.685	0.086	0.123	0.523	0.483	0.040	0.082
<i>Gpi</i>	0.304	0.294	0.010	0.037	0.264	0.253	0.011	0.047
Mean	0.233	0.212	0.021	0.100	0.172	0.165	0.007	0.045

Figure 1 PCR-fingerprinting of genomic DNA of *P. eryngii* and *P. ferulae* with minisatellite M13. Lanes 1, 16 100 bp molecular-weight marker. Lanes 2–8, 10, 14 *P. eryngii*; lanes 9, 11–13, *P. ferulae*; lane 15 control without template DNA.

populations differed greatly among loci and were highest for *Dia* ($G'_{ST}=0.241$), *Ap-2* ($G'_{ST}=0.058$), *Mpi* ($G'_{ST}=0.123$) in *P. eryngii*, and for *Mpi* ($G'_{ST}=0.082$), *Idh-2* ($G'_{ST}=0.072$) and *Pgm* ($G'_{ST}=0.080$) in *P. ferulae*. However, the locus *Pep-2* was fixed for an allele within *P. ferulae* populations. While the largest proportion of genetic diversity was because of variation within populations, $H_S=0.212$ in *P. eryngii* and $H_S=0.165$ in *P. ferulae*, the remaining variation, $D_{ST}=0.021$ and 0.007 , was because of allele frequency differences between populations.

The gene flow (Nm) estimates obtained for each locus by the F_{ST} (Nm_{FST}) were in *P. eryngii* ($Nm_{FST}=3.50$) and in *P. ferulae* ($Nm_{FST}=5.43$). The different estimates of gene flow observed between the two taxa may be because of the lesser number of samples of *P. eryngii* compared to *P. ferulae*.

PCR-fingerprinting

Genetic polymorphism: The minisatellite M13 amplified 2217 scored bands in the 81 specimens of *Pleurotus eryngii* (populations 2, 3, 5 and 6) and 87 specimens of *P. ferulae*

(9, 12, 15 and 19). Of these, only well-amplified (sharp gel signal) and reproducible (in all experiments) bands were considered for the analysis (Figure 1). The number of bands obtained varied from 4 to 13 for each specimen. Three bands appeared to discriminate the two taxa: *P. eryngii* were characterized by a band at 1140 bp, while *P. ferulae* showed two bands at 300 and 1200 bp (Figure 1). The analysis of banding patterns revealed polymorphism both between and within populations of the two taxa. A similar number of specific bands (11–15) was found in *P. eryngii* and *P. ferulae* populations. The level of intrapopulation polymorphism varied from one population to another. None of the *P. eryngii* and *P. ferulae* populations were completely monomorphic; the polymorphic bands varied from 30 to 50% within *P. eryngii* and from 40 to 50% within *P. ferulae* populations (Table 4). These differences were not significant between taxa.

Genetic diversity: AMOVA for the two taxa indicated significant genetic differences ($P<0.0005$) within populations, but no significant difference was observed between regions (Table 5). In *P. eryngii* the total genetic diversity was ascribed to individual differences within populations (61.3%), to population differences within regions

Table 4 Frequency of polymorphic PCR fingerprinting bands in *P. eryngii* (2, 3, 5, 6) and *P. ferulae* (9, 12, 15, 19) populations. *P* is the proportion of polymorphic fragments

Code	Populations	Polymorphic fragments amplified with primer M13																			<i>P</i>
		<i>N</i>	300	400	450	500	530	600	640	700	800	850	900	1000	1140	1200	1300	1500	1700	2000	
2	Castel del Monte	18	0	0.2	0	0	1	0.5	0.7	0.2	1	0.2	1	1	1	0	1	0	0	0	0.3
3	Ceglie Messapico	18	0	0.3	0.5	0.3	1	1	0.3	0.5	1	0	1	1	1	0	1	0	0	0	0.3
5	Mamone	20	0	0	0.5	0.1	1	0.8	0.5	0.8	1	0.5	1	0.8	1	0	1	0	0.6	0.3	0.5
6	Macomer	25	0	0	0	0	1	1	0.8	0.3	0.8	0.8	1	1	1	0	1	0	1	0.3	0.3
9	Castel del Monte	21	1	0	0	0	0.8	0.8	0.7	0	0.3	0.2	1	0.5	0	1	1	0	0.2	0	0.4
12	Mamone	20	1	0	0.8	1	1	0.3	0.7	0	0.8	0	1	0	0	1	0.8	0	0.5	0.7	0.4
15	Macomer	22	1	0	0	0.4	1	0.3	0.4	1	1	0.3	1	0.6	0	1	1	0.4	0.4	0.6	0.5
19	Paulilatino	24	1	0	0.3	0.1	1	0.6	0.6	0.4	0.8	0	1	0.4	0	1	1	0	0.4	0.1	0.5

Table 5 Analysis of molecular variance (AMOVA) for 81 specimens of *P. eryngii* and 87 specimens of *P. ferulae*

Source of variation	<i>Pleurotus eryngii</i>							<i>Pleurotus ferulae</i>						
	<i>df</i>	<i>SSD</i>	<i>MSD</i>	Variance components	% Total variance	<i>P</i>	Φ statistics	<i>df</i>	<i>SSD</i>	<i>MSD</i>	Variance components	% Total variance	<i>P</i>	Φ statistics
Global														
Between regions	1	9.621	9.621	0.397	17.16	=0.194	0.172	1	5.542	5.542	0.125	4.46	=0.184	0.045
Between populations	2	9.443	4.722	0.498	21.51	=0.065	0.260	2	9.196	4.598	0.321	11.40	=0.091	0.119
Within populations	23	32.639	1.419	1.420	61.33	<0.0005	0.387	23	54.446	2.367	2.367	84.13	<0.005	0.159
Within regions														
Between populations	3	19.065	6.355	0.751	34.62	<0.01		3	14.739	4.913	0.379	13.80	<0.01	
Within populations	23	32.639	1.419	1.419	65.38	<0.0005		23	54.446	2.367	2.367	86.20	<0.0005	
Between regions														
Between regions	1	9.621	9.621	0.630	27.25	=0.07		1	5.542	5.542	0.321	11.20	=0.065	
Within regions	25	42.082	1.683	1.683	72.75	<0.0005		25	63.642	2.546	2.546	88.80	<0.0005	

df-degrees of freedom; *SSD*-sums of squared deviations from the mean; *MSD*-mean squares deviations; *P*-variance components significance

(21.5%) and to regional divergence (17.2%). Most of the genetic variation observed within regions was because of differences within populations (65.4%), and the genetic variation between regions was accounted for by the variation within regions (72.8%). The total genetic diversity in *P. ferulae* was mostly because of variations within populations (84.1%), as compared with variation within regions (11.4%) and with regional divergence (4.5%). Furthermore, genetic variation within and between regions was ascribed to individual differences (86.2 and 88.8%, respectively). Both approaches, allozymes and PCR-fingerprinting, yield an overall similar trend; genetic diversity is greater within than among populations.

Discussion

The principal aim of this study was to document the level and distribution of genetic diversity in Italian populations of two *Pleurotus* species, *P. eryngii* and *P. ferulae*.

The level of genetic variation was evaluated by PCR-fingerprinting and allozyme analyses in these populations. With PCR-fingerprinting, the frequencies of polymorphic bands were not significantly different between

P. eryngii and *P. ferulae* populations. The values of the genetic parameters with allozymes were lower ($P=0.45$, $H_o=0.161$ in *P. eryngii* and $P=0.40$, $H_o=0.117$ in *P. ferulae*) than the values observed in some basidiomycetes (eg in *Mycena rosea*, $H_o=0.217$, Boisselier-Dubayle et al, 1996; *Agaricus campestris* $H_o=0.281$, May and Rose, 1982). Similar values of genotypic diversity were recorded by Zervakis et al (1994) in *Pleurotus* species: *P. ostreatus* (0.093), *P. pulmonarius* (0.083) and for *P. eryngii* complex (0.137). The comparison with the above results is somehow problematic since the authors derived their values from either few isolates or few gene-enzyme systems or both, so they might not be strictly equivalent to our results. High levels of genetic variation are generally expected for wild organisms that reproduce sexually and have broad ecological niches and a wide geographical distribution (Babbel and Selander, 1974; Hamrick and Godt, 1990; James et al, 1999). By contrast, low levels of variation were observed in species with a restricted distribution, or that have long been cultivated for commercial purposes, or in populations with just a few individuals, or in species that reproduce exclusively asexually (Old et al, 1984; Burdon and Roelfs, 1985). For example, high levels of genetic variation were observed in broadly distributed species, such as *Schizophyllum*

commune (James *et al*, 1999). Nevertheless, high genetic variation has been observed in species such as *Mycena rosea*, with narrow distributions (only in Western Europe) (Boisselier-Dubayle *et al*, 1996). High genetic variability would therefore seem attributable to species of wild origin, which reproduce sexually. *Pleurotus eryngii* and *P. ferulae* have both these characteristics. They are distributed throughout rather circumscribed areas and are related to specific host plants, *E. campestre* and *F. communis*, respectively, which could lead to the values of genetic variability observed within populations of the two species. Our results could fit with Chen *et al* (1992) hypothesis, which ascribed the moderate level of genetic variation in the fungi of the genus *Pythium* to selection pressures consistent with host-specific evolutionary development. This hypothesis will remain as such until a deeper knowledge of the degree of genetic variation in fungi is acquired.

Heterozygote deficiency within some populations of the two species was found at few loci tested. Since inbreeding is revealed through heterozygote deficit, it seems to have little influence on the populations of the two taxa. In fact the inbreeding levels observed were not high ($F_{IS}=0.235$ in *P. eryngii* and 0.262 in *P. ferulae* populations). High inbreeding coefficients usually range between 0.80 and 1.00, and are found in species where inbreeding prevails. Conversely, outcrossing values were higher than inbreeding coefficients, and ranged from 0.485 to 0.914 in *P. eryngii* and from 0.416 to 0.721 in *P. ferulae*. This would demonstrate that mechanisms that restrain inbreeding are acting at the local level, in agreement with Bresinsky *et al* (1987) results on the genus *Pleurotus*. With crossbreeding experiments they demonstrated that both inbreeding and outcrossing are possible; however, outcrossing can be favored by the multiple allelism of the mating factors A and B.

The estimates of genetic differentiation, averaged over all loci, indicated that the total genetic diversity was low ($G_{ST}=0.100$ in *P. eryngii* and $G_{ST}=0.045$ in *P. ferulae*), and a high level of variation was found within populations ($H_S=0.212$ in *P. eryngii* and $H_S=0.165$ in *P. ferulae*). Similar results were obtained by AMOVA analysis of the populations, which demonstrated that a great portion of the total genetic variation could be attributed to the differences within populations, revealing low genetic differentiation over moderate (within regions) and large geographic distances (among regions).

Partitioning of genetic variation within, rather than among, populations has been observed also in other Basidiomycete species. Mating system and gene flow are some of the most important factors that determine genetic structure. Some studies have led to the generalization that outcrossing species maintain most genetic variability within, rather than among, populations, whereas inbreeding species partition most variability among populations (James *et al*, 1999). The gene flow, by spore dispersal, appears to be another major force in the determination of the local genetic structure of populations. Several studies on lignicolous, humicolous and saprotrophic fungi have shown that, when the local populations are maintained chiefly by basidiospore dispersal, several genetically different individuals occupy a discrete zone within a substrate: *Pleurotus ostreatus* (Kay and Vilgalys, 1992); *Mycena rosea* (Boisselier-Dubayle *et al*,

1996); *Collybia subnuda* and *Marasmiellus praecutus* (Murphy and Miller, 1993). In contrast, the populations maintained by basidiospore dispersal and subsequent rapid vegetative spread on nearby substrates consist of few individuals or just one individual with genetically identical clones: *Armillaria bulbosa* (Smith *et al*, 1992); *Lycoperdon pyriforme* (Huff, 1993). In this case the transition to a new substrate may occur not by the arrival of spores directly to the substrate, but from colonies initially established in the leaf litter or soil. These two different dispersal processes in fungal populations could reflect the different genetic structure of the populations: low genetic diversity and high gene flow between populations were observed in those populations that are primarily maintained through basidiospore dispersal.

The data we present show a similar genetic diversity partitioning pattern for *P. eryngii* and *P. ferulae*. Most of the observed variation is contained within local populations, which is likely to be the result of high gene flow, by efficient patterns of dispersal in sexual, outcrossing species. The high values of gene flow observed in *P. eryngii* ($Nm_{FST}=3.50$) and *P. ferulae* ($Nm_{FST}=5.43$), and the outcrossing mechanisms that restrain inbreeding seem to play an important role in the partitioning of genetic diversity of the Italian populations of *P. eryngii* and *P. ferulae*. Our findings agree with the results obtained by Boisselier-Dubayle *et al* (1996) for the saprophytic fungus *Mycena rosea* and would indicate basidiospore dispersal as a primary mechanism for dykaron establishment in the two species. Further research into the spatial distribution and genetic relationships among individuals will be necessary to confirm our conclusions.

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