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Diversity and spatial distribution of vegetative compatibility types and mating types of *Cryphonectria parasitica* in the Aydın Mountains, Turkey

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Abstract In this study, the population structure of the chestnut blight fungus *Cryphonectria parasitica* in the Aydın Mountains was investigated to make inferences about fungal reproduction and population diversity. A total of 213 *C. parasitica* isolates from eight subpopulations were used to determine vegetative compatibility (vc) and mating types of the population. Furthermore geostatistical analysis was performed to define the spatial structure of the population. The results showed that the isolates were vegetatively compatible with the European vc types of either EU-1 or EU-12. Both vc types were found in almost all subpopulations, but their frequencies varied depending on location. The results of a PCR assay showed that both mating types of *C. parasitica* (*MAT-1* and *MAT-2*) exist in the population. *MAT-1* comprised 65% of the total isolates, and the ratio of mating types was significantly skewed from 1:1. Genotyping based on combined vc and mating type data revealed four genotypes: *EU-1/MAT-1* (28.6%), *EU-1/MAT-2* (34.7%), *EU-12/MAT-1* (36.2%) and *EU-12/MAT-2* (0.5%). Geostatistatical analysis indicated that vc types, mating types and vc/mating genotypes were spatially autocorrelated and clustered in their distributions. Results suggested that *C. parasitica* could have a clonal population structure that is generated by asexual reproduction. Low vc-type diversity suggests that the *C. parasitica* population in the Aydın Mountains may be highly suitable to hypovirus invasion, thereby providing a high potential for successful biological control. However, co-occurrence of sexually compatible strains of EU-1 and EU-12 at the same locations in close proximity creates a high risk of increase in vc-type diversity.

Keywords Chestnut · Chesnut blight · Geostatistics · Hypovirulence · Kriging · Population analysis

Introduction

Chestnut blight, caused by *Cryphonectria parasitica* (Murrill) Barr., is one of the most important diseases of chestnut worldwide (Anagnostakis 1987; Robin and Heiniger 2001). Since its discovery in New York in 1904, billions of chestnut trees were reported to have died from this disease both in America and Europe over the past century (Anagnostakis 1987; Robin and Heiniger 2001). In Turkey, the disease was first reported in the Marmara Region in 1967 (Akdoğan and Erkman 1968). Since then, incidence of the disease has progressively increased, and it has

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spread throughout the other chestnut-growing areas of Turkey such as the Black Sea and Aegean regions (Gürer et al. 2001; Çeliker and Onoğur 2001; Erincik et al. 2003).

Studies have shown that using hypovirulent strains in biological control of the pathogen is the most efficient control method to date (Anagnostakis 2001; Robin and Heiniger 2001). Hypovirulence is a phenomenon that occurs when *C. parasitica* is infected by double-stranded RNA (dsRNA) hypoviruses, which reduces its ability to colonize the inner bark and kill the tree (Anagnostakis 2001; Robin and Heiniger 2001). Either artificial introductions or natural occurrences of hypovirulence resulted in recovery of many chestnut stands from chestnut blight in Europe, as well as in the United States (Anagnostakis 2001; Robin and Heiniger 2001). Hypovirulence is transmissible in the cytoplasm between *C. parasitica* strains via hyphal anastomosis that is governed by at least six vegetative incompatibility (*vic*) loci (Cortesi et al. 2001; Biella et al. 2002). It is well established that hypoviruses are easily transmitted from hypovirulent strains to virulent ones if the donor and recipient are the same at all *vic* loci (Cortesi and Milgroom 1998; Cortesi et al. 2001). By contrast, virus transmission is restricted or occurs at significantly lower frequencies between individuals that each carry different *vic* alleles (Cortesi et al. 2001; Biella et al. 2002).

Inefficient biological control of chestnut blight in certain locations in the North America has been associated with high vc-type diversity of *C. parasitica* (Anagnostakis et al. 1986; Liu and Milgroom 1996). Increases in vc-type diversity are predominantly generated by sexual reproduction because more vc types arise through the recombination of *vic* genes (Milgroom 1995; Cortesi and Milgroom 1998). Sexual reproduction in *C. parasitica* is genetically governed by *MAT-1* and *MAT-2* mating alleles (Marra and Milgroom 2001). Although self-fertilization preferably occurs in heterokaryotic strains carrying both alleles, *C. parasitica* is a heterothallic fungus; i.e., mating only takes place between isolates that have different *MAT* alleles (Marra and Milgroom 2001).

GIS and geostatistics have recently started to be used in plant pathology in order to make inferences about how plant pathogens spread and how their population structure changes over space and time (Jaime-Garcia et al. 2001; Dutech et al. 2008). *C. parasitica* may exhibit various spatial distribution patterns depending on the types of inoculum produced (Milgroom and Lipari 1995; Liu et al. 2000; Dutech et al. 2008). The fungus can produce inoculum either sexually as ascospores or asexually as conidia (Milgroom et al. 1991). Ascospores are dispersed long distances by wind, thereby producing a random distribution pattern. By contrast, conidia are splash-dispersed and are carried by rain or animals over short distances, thus forming a clustered pattern (Liu et al. 2000). Variogram analysis in geostatistics is used for measuring the degree of spatial dependence (spatial autocorrelation) between individuals; i.e., it provides a description of how individuals are correlated with distance (Jaime-Garcia et al. 2001; Dutech et al. 2008). For patterned data with positive spatial autocorrelation, variance increases with distance, which can be interpreted as follows: samples that are close together are more similar than samples that are farther apart. Kriging is another geostatistical technique that is used to estimate values for unsampled points by interpolation and to create surface maps to visualize spatial patterns (Jaime-Garcia et al. 2001; Durdu 2009).

The Aydın Mountains form a natural boundary between the Aydın and İzmir provinces in the Aegean Region and hold approximately 40% of Turkey's chestnut production. Chestnut blight was first reported at a northern site in the mountains (İzmir) in 2001 (Çeliker and Onoğur 2001) and at a southern site (Aydın) in 2003 (Erincik et al. 2003). In previous studies conducted in the area, two vc types (EU-1 and EU-12) and two mating types (*MAT-1* and *MAT-2*) of *C. parasitica* were detected (Çeliker and Onoğur 2001; Erincik et al. 2007). However, no perithecia have been observed in the Aydın Povince (Erincik et al. 2007). The low *vc*-type diversity in the province may provide an ideal condition for the efficient spread of natural or introduced hypovirulence, but the sustainability of this condition mostly depends on the absence of sexual crosses between two different vc types. Since EU-12 and EU-1 are genetically different from each other at four *vic* loci, 14 nonparenthal vc types are expected to be generated after the recombination of these vc types (Cortesi and Milgroom 1998). Therefore, the population structure of *C. parasitica* and the spatial distribution of vc and mating types of this pathogen should be taken into account during the introduction of hypovirulent strains to the environment.

The objectives of this study are to determine vc and mating-type diversity of *C. parasitica* within a broader population with a more fine-scale perspective than obtained previously in the Aydın Mountains, and to analyze the spatial pattern of these vc and mating types using geostatistics.

Materials and methods

Sampling and isolation

The study was conducted in six counties: Nazilli, Köşk, Sultanhisar, Central County, Beydağ, and Ödemiş in the Aydın and İzmir provinces located in the Aydın Mountains (N37°58′-E27°58′; N38°06′-E28°28′) in 2006–2008 (Fig. 1). Since Nazilli and Ödemiş counties have larger study areas compared to the others, each was divided into two sub-regions as follows: Nazilli-1 (East-Nazilli); Nazilli-2 (West-Nazilli); Ödemiş-1 (East-Ödemiş) and Ödemiş-2 (West-Ödemiş). *C. parasitica* isolates were sampled at 41 different sampling spots, each consisting of a circle 500 m in diameter. Depending on the blight incidence (*visual observation*) at the sampling spot, the number of randomly selected samples varied from two to fifteen. The distances between neighboring samples varied from 6 to 400 m. During each sampling, a piece of bark tissue (5×5 cm) was removed from the margin of an actively growing canker by a surface-disinfected knife. Geographical coordinates for each sample were recorded using a handheld GPS device (Model Etrex Vista, Garmin International Inc.). *C. parasitica* was isolated from the samples using standard isolation techniques. One isolate was retained per sample. In total, 213 *C. parasitica* isolates were cultured. Among the isolates, none of them exhibited white mycelial growth in culture media; therefore, no study was conducted on hypovirulence.

Vegetative compatibility test and analysis of vc-type diversity

Vegetative compatibility types of the isolates were determined by pairing each isolate with European vc tester isolates (obtained from Prof. Paolo Cortesi, Università degli Studi di Milano, Dip. di Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversità, Milano, Italy) on PDA (Erincik et al. 2007). The diversity of vc types in

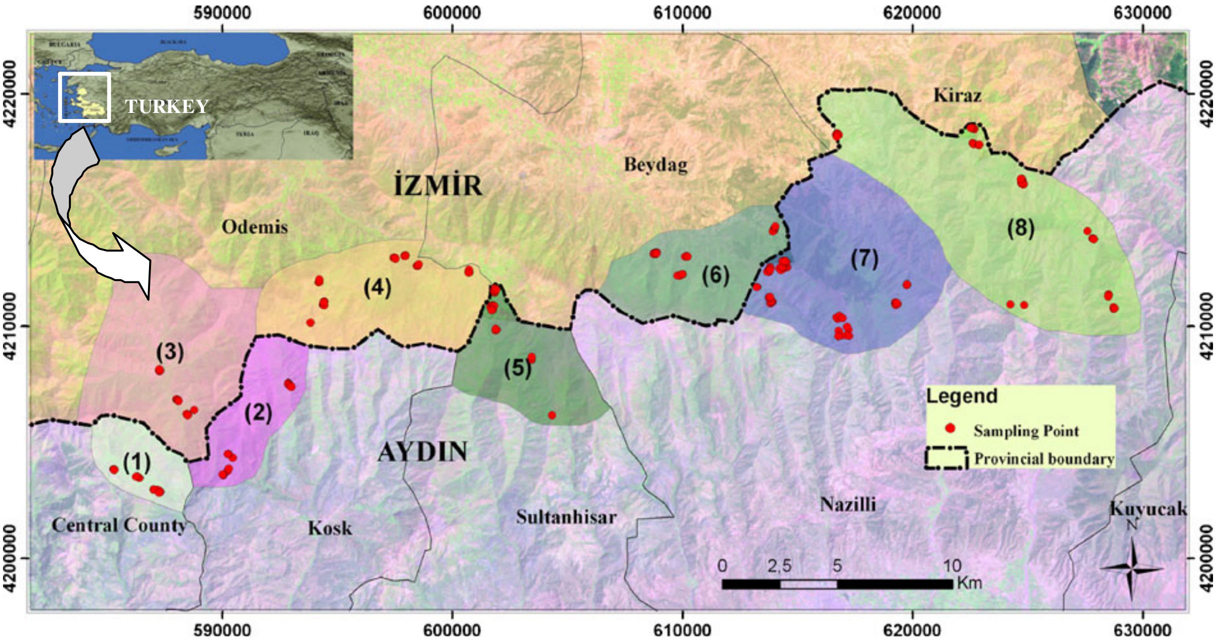

Fig. 1 Map of study area showing the locations where *Cryphonectria parasitica* subpopulations were collected. Sampling locations: Central County (*1*), Köşk (*2*), Ödemiş-2 (*3*), Ödemiş-1, (*4*), Sultanhisar (*5*), Beydağ (*6*), Nazilli-2 (*7*), Nazilli-1 (*8*)

the populations was evaluated using richness, a diversity index, and evenness (Grünwald et al. 2003). Richness (*S*) is the number of observed vc types in each subpopulation. The Shannon Wiener Index (H') was used for quantifying diversity. H' was calculated as: $-\sum p_i \ln p_i$, where p_i was the frequency of the *i*th vc type. The distribution of vc types within the samples was measured by evenness, which was calculated using the following equation: $E_5 = (1/\sum p_i^2 - 1)/(e^{H'} - 1)$ (Grünwald et al. 2003).

Mating type assay

Mating type of each isolate was determined by a multiplex polymerase chain reaction (MPCR) assay, which was performed with specific primers M1-GS1n and M1-GS3-rev for *MAT-1* and M2-GS3 and gs1-d-1 for *MAT-2* (Marra and Milgroom 2001; McGuire et al. 2004). DNA isolation and PCR amplification were carried out as described previously by Marra and Milgroom (2001). Amplified DNA fragments were separated by electrophoreses in 1% (*w/v*) agarose gel at 60 V for 1 h. As a molecular size marker, 1 kb DNA Ladder (Fermentas) was used. After ethidium bromide staining, amplification products were visualized under a transulliminator. The DNA samples for each mating type isolate were used as references in PCR assays. Mating types were determined according to the size of the amplification product using agarose gel electrophoresis.

Using chi-squared statistics, ratios of mating-type alleles were tested for deviation from equal frequencies of the two mating types (1:1), which is expected in sexually reproducing populations (Bissegger et al. 1997)

Geostatistical analysis

Geostatistical analysis was performed using kriging methodology and variogram analysis. Geographical coordinates of the observed data were processed using MapSource 6 (Garmin International Inc.). In the kriging analysis, ordinary kriging was utilized for processing the observed data. Since all variables were categorical, vc types, mating types and vc/mating genotypes were transformed into indicator variables by scoring them as 1 or 0, depending on the presence or absence of the character, respectively (Jaime-Garcia et al. 2001). Ordinary kriging provided surface maps of the probability of occurrences of vc types, mating types and vc/mating genotypes, which helped in the visualization of the spatial distribution patterns. In addition, in order to determine high-risk areas for sexual reproduction (and thereby areas where new vc types of *C. parasitica* were likely to be generated), 41 sampling spots were also analyzed in the kriging analysis by scoring the spots where mating was possible as 1 (i.e., sexually compatible isolates of EU-1 and EU-12 were present) and the spots where mating was not possible as 0. Kriging analysis was conducted using ArcGIS 9.2 (ESRI) software.

In order to characterize the variance structure of each vc type, mating type and vc/mating genotypes over the study area, a variogram analysis was performed (Jaime-Garcia et al. 2001). In the variogram analysis, the following spherical-type variogram model was used:

$$\gamma(h) = C_o + C\left[\frac{3h}{2a} - \frac{h^3}{2a^3}\right],\ for\, 0 < h \leq a$$
$$\rightarrow \gamma(h) = C_o + C, for\, h > a \qquad (1)$$

where h is the average separation distance between sample pairs, C_o is the nugget, C is the structural variance, C_o+C is the sill, and a is the range. The range, nugget and sill are the parameters describing a variogram model (Durdu 2009). The experimental variogram values increase with increasing distance until reaching the maximum (C) at a distance known as the range (a). If at a distance nearly equal to zero the variogram value is greater than zero, this value is known as the nugget effect (C_o). The *sill* (C_o+C) is the overall sample variance at the range (Durdu 2009). Variances computed from omnidirectional experimental variograms are plotted versus lag distances as a variogram using VARIOWIN 2.1 software (Pannatier 1994).

Results

Diversity of vc types

In the vegetative compatibility test, all *C. parasitica* isolates were compatible with either EU-1 or EU-12 (Table 1). Both vc type were found in nearly all subpopulations except Köşk, where EU-1 was the

Table 1 Diversity of vegetative compatibility (vc) types, mating types and vc/mating genotypes of *Cryphonectria parasitica* in the Aydın Mountains

Province	Subpopulation	N[a]	Genotypes[b]				Vc-type diversity			Mating-type diversity		
			EU-1/MAT-1	*EU-1/MAT-2*	*EU-12/MAT-1*	*EU-12/MAT-2*	S (g_{obs})[c]	H'[d]	E_5[e]	Mating type ratio (*MAT-1:MAT-2*)	χ^2[f]	P[g]
Aydın												
	Nazilli-2 (West)	54	26	13	15		2	0.59	0.83	41:13	14.51	<0.001
	Nazilli-1 (East)	44	10	33	1		2	0.11	0.41	11:33	11.00	<0.001
	Köşk	16	2	14	0		1	0.00		2:14	9.00	<0.01
	Sultanhisar	22	7	0	14	1	2	0.63	0.88	21:1	18.18	<0.001
	Central County	14	7	4	3		2	0.52	0.75	10:4	2.57	>0.05
	Subtotal	150	52(34.7%)	64(42.7%)	33(22.0%)	1(0.6%)	2	0.54	0.76	85:65	2.67	>0.05
İzmir												
	Ödemiş-1(East)	25	2	0	23		2	0.28	0.54	25:0	25.00	<0.001
	Ödemiş-2 (West)	14	0	4	10		2	0.60	0.84	10:4	2.57	>0.05
	Beydağ	24	7	6	11		2	0.69	0.99	18:6	6.00	<0.05
	Subtotal	63	9(14.3%)	10(15.9%)	44(69.8%)		2	0.61	0.86	53:10	29.35	<0.001
Total		213	61(28.6%)	74(34.7%)	77(36.2%)	1(0.5%)	2	0.66	0.93	138:75	18.63	<0.001

[a] Number of isolates in each subpopulation

[b] Genotyping based on a combination of mating and vegetative compatibility traits. Numbers in parentheses indicate percentages of each genotype in the population

[c] S (Richness): number of vc types found in each subpopulation

[d] Shannon diversity index: $H' = -\Sigma p_i \ln p_i$, where pi is the frequency of the ith vc type

[e] Evenness: $E_5 = (1/\sum p_i^2 - 1)/(e^{H'} - 1)$

[f] Chi-square test

[g] P value from χ^2 test for deviation from 1:1 ratio

unique vc type. An apparent difference was observed between the southern site (Aydın Province) and the northern site (İzmir Province) of the Aydın Mountains in terms of vc-type distribution. In the southern site, EU-1 was dominant in four of the five subpopulations of Aydın Province and comprised 77% of 150 isolates. By contrast, in the northern site, EU-12 was dominant in two of the three subpopulations of İzmir Province and comprised of 70% of 63 isolates.

Shannon Diversity Index (H') for the isolates from eight subpopulations, in terms of vc type, ranged from 0 to 0.69 (Table 1). Only one subpopulation from Aydın (Köşk) exhibited a lack of diversity with one vc type. The highest index value (0.69), corresponding to the most diverse population, was found in Beydağ. Nazilli-1 (East-Nazilli) and Ödemiş-1 (East-Ödemiş) subpopulations had lower diversity indices compared to the others. Evenness for vc types ranged from 0.41 to 0.99. Beydağ and Nazilli-1 were the most and least evenly distributed subpopulations, respectively (Table 1).

Distribution of mating types

Multiplex PCR assay results showed that both *MAT-1* and *MAT-2* mating types of *C. parasitica* were present in the Aydın Mountains (Table 1). Out of 213 isolates, 138 were *MAT-1* comprising 65% of *C. parasitica* isolates (Table 1). A chi-square test showed that the overall mating-type ratio was highly significantly skewed from 1:1, which is not expected in sexually reproducing populations. However, for the isolates from Aydın, the ratio of *MAT-1* to *MAT-2* was 85:65 and not significantly skewed from 1:1. On the other hand, analysis of the data at a finer spatial scale revealed that almost all subpopulations in this province, except in the Central County, had mating ratios that were significantly skewed from 1:1. *MAT-1* was dominant in Nazilli-2 (West-Nazilli) and Sultanhisar, whereas *MAT*-2 was dominant in Nazilli-1 and Köşk. In İzmir, *MAT-1* was dominant and comprised 84% of 63 isolates. All isolates were *MAT-1* in Ödemiş-1, whereas the mating-type ratio was close to 1:1 in Ödemiş-2 (West-Ödemiş).

Genotyping based on mating and vegetative compatibility traits

Evaluation of the isolates based on a combination of mating and vc traits showed that the *C. parasitica* population in the Aydın Mountains consisted of the following four genotypes: *EU-1/MAT-1*, *EU-1/MAT-2*, *EU-12/MAT-1* and *EU-12/MAT-2* (Table 1). The percentages of *EU-1/MAT-1*, *EU-1/MAT-2*, *EU-12/MAT-1* and *EU-12/MAT-2* in the total population were 28.6%, 34.7%, 36.2%, and 0.5% respectively. Four subpopulations (Nazilli-1, Nazilli-2, Central County and Beydağ) had a mixture of *EU-1/MAT-1*, *EU-1/MAT-2* and *EU-12/MAT-1* genotypes. In these subpopulations, *EU-12/MAT-1* and *EU-1/MAT-2*, which are the sexually compatible genotypes that have the potential to generate new (nonparental) vc types from sexual crossing, were observed together in six sampling spots. The minimum distances between *EU-12/MAT-1* and *EU-1/MAT-2* isolates in these spots ranged from 25 to 83 m (± 7–14 m) (data not shown). On the other hand, *EU-1/MAT-1* and *EU-12/MAT-2*, which are the other genotypes that have the potential to generate new vc types, were found together in only one sampling spot in Sultanhisar County.

Geostatistical analysis

The probability maps of vc types and mating types of *C. parasitica* derived from indicator kriging are demonstrated in Fig. 2. These maps illustrate the probability of occurrence of each vc type and mating type in the Aydın Mountains. Figure 2a shows that vc type EU-1 had the highest probability of occurrence (>0.65) in the eastern and central parts of Nazilli, the eastern part of Beydağ, Köşk and Central County. The other vc type, EU-12, had the highest probability of occurrence (>0.65) in the southwestern parts of Beydağ Sultanhisar and Ödemiş (Fig. 2b). Mating type *MAT-1* had the highest probabilities of occurrence (>0.65) in Ödemiş, southwestern parts of Beydağ, Sultanhisar, and in scattered small areas in Nazilli and Central County (Fig. 2c). *MAT-2* had the highest probabilities of occurrence (>0.65) in the northeastern part of Nazilli and the central part of Köşk (Fig. 2d). The maps also show some small scattered areas where the probability of occurence for both mating types was between 0.35 and 0.65. *C. parasitica* populations in such areas have the potential to reproducing sexually.

The probability maps of four *C. parasitica* genotypes based on a combination of vc and mating traits are shown in Fig. 3. The maps clearly illustrated that each genotype is centered in different locations. The genotype *EU-1/MAT-1* had the highest probability of occurrence (>0.65) mainly in the southern hills of the Aydın Mountains (Aydın Province), such as West-Nazilli and Central County (Fig. 3a). The *EU-1/MAT-2* genotype had the highest probabilities of occurrence (>0.65) in two centres located in eastern Nazilli and Köşk (Fig. 3b). The probability of occurrence of the *EU-12/MAT-1* genotype was higher mainly in the northern hills of the Mountains (Ödemiş and Beydağ Counties) (Fig. 3c). The probability of occurrence of the *EU-12/MAT-2* genotype was estimated to be zero in nearly all of the study area because only one isolate of this genotype was found (data not shown).

Figure 4 shows the probability of the *MAT-1* and *MAT-2* isolates occurring together, which indicates risky areas where sexual mating in *C. parasitica* can take place. Nazilli and Beydağ, two neighbouring counties, had the largest risky area; here, the probability of occurrence was higher than 0.65 (Fig. 4a). However, there were also some small risky areas in Central County, Köşk and Sultanhisar where the probabilities were higher than 0.65. Figure 4b shows the probability of the concurrent presence of sexually compatible vc/mating genotypes (*EU-1/MAT-2* vs *EU-12/MAT-1* or *EU-1/MAT-1* vs *EU-12/MAT-2*), which have the potential to generate nonparental vc types from sexual mating. In the majority of the Mountains, this probability was found to be lower than 0.35 (Fig. 4b). However, there were several large areas in Nazilli and Beydağ counties with probabilities that were higher than 0.65, which indicates the most risky areas for the generation of nonparental vc types. Two more smaller risky areas were also found in Sultanhisar and Central counties.

As shown in Fig. 5, the experimental variograms for vc type, mating type and vc/mating genotype increased progressively with distance and exhibited random fluctuation around a sill value beyond the

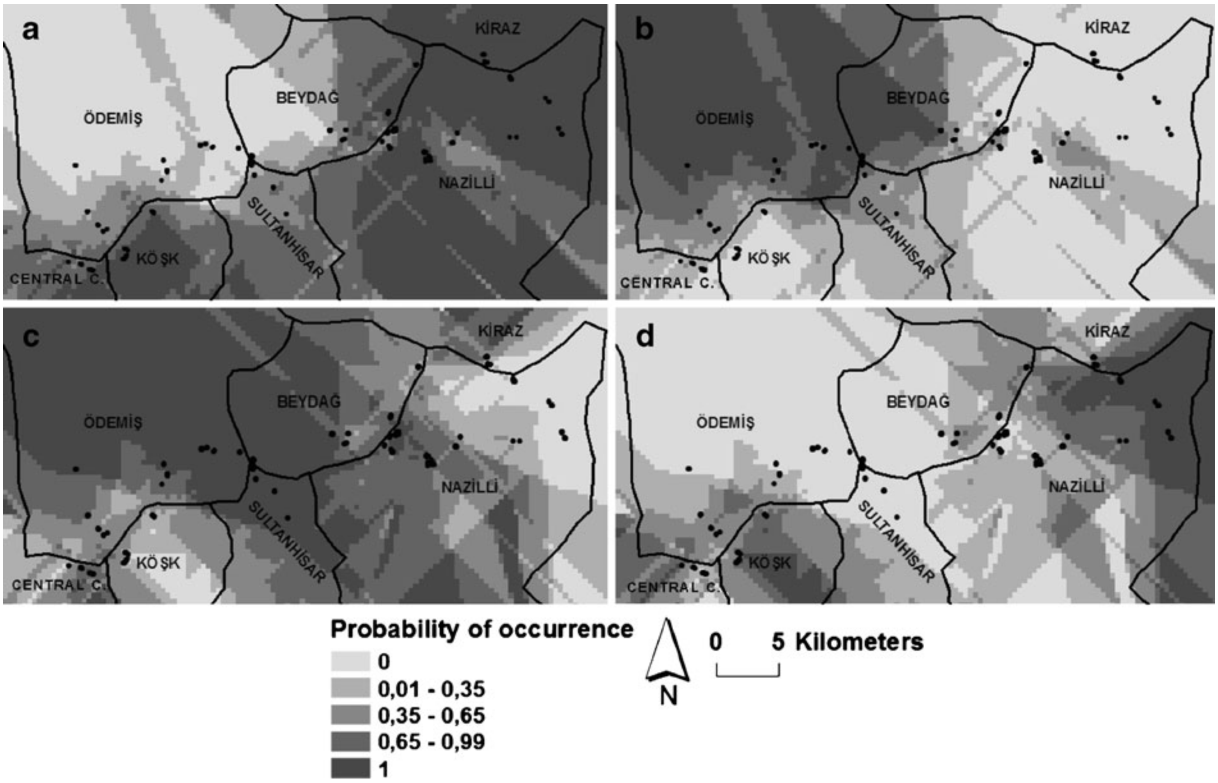

Fig. 2 Probability of occurrence of *vc* types and mating types of *Cryphonectria parasitica* in the Aydın Mountains based on indicator kriging; **a** vc type EU-1; **b** vc type EU-12; **c** mating type *MAT-1*; and **d** mating type *MAT-2*. *Dots* represent the sampling points

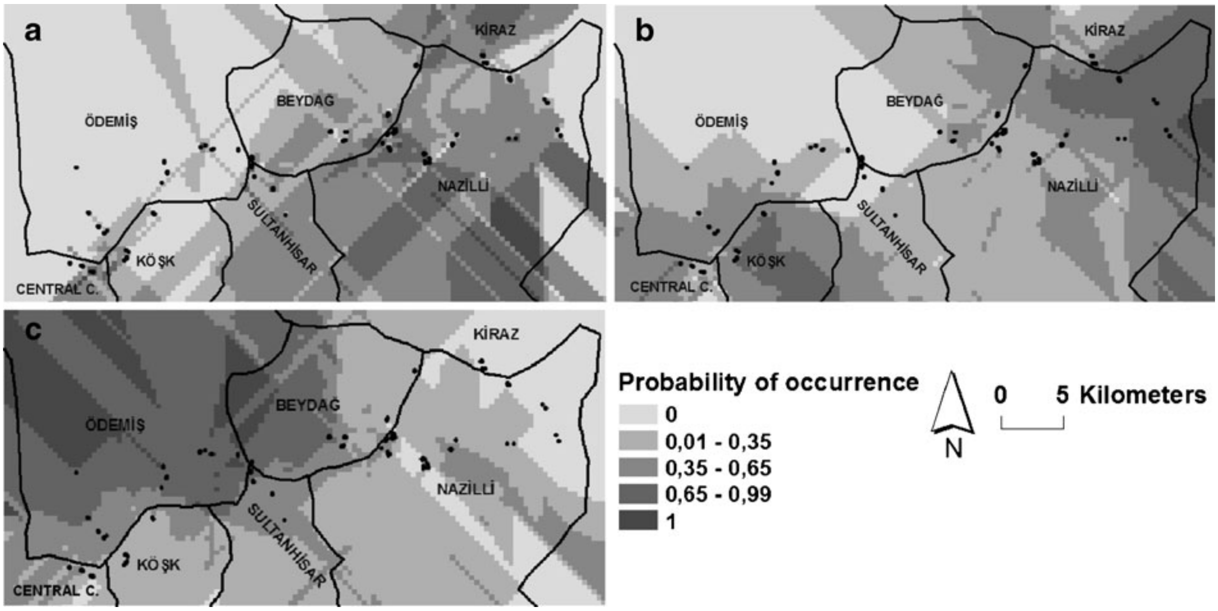

Fig. 3 Probability of occurrence for four genotypes of *Cryphonectria parasitica* based on vegetative compatibility and mating traits in the Aydın Mountains derived from indicator kriging; **a** *EU-1/MAT-1*; **b** *EU-1/MAT-2*; and **c** *EU-12/MAT-1*. *Dots* represent sampling points

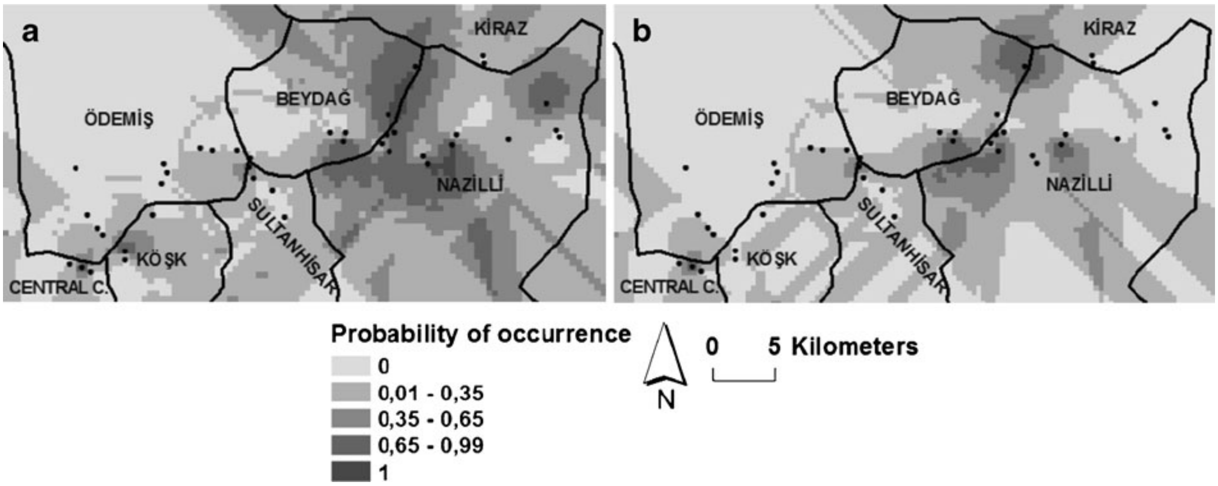

Fig. 4 **a** Probability of occurrence of concurrent presence of *MAT-1* and *MAT-2* isolates of *Cryphonectria parasitica* in the same sampling spots based on indicator kriging; **b** Probability of occurrence of concurrent presence of sexually compatible EU-1 and EU-12 isolates of *C. parasitica* in the same sampling spots based on indicator kriging. *Dots* represent sampling spots

range of influence. These results imply that a spherical model can obtain a good fit. All variables were spatially autocorrelated with model variograms. Table 2 presents summaries of variograms for vc type, mating type and vc/mating genotype. The spatial autocorrelation extended within the ranges of 6160 m for vc type, 7,920 m for mating type, and 3,520 m for vc/mating genotype. Samples separated by a distance less than these ranges were related to each other, whereas samples at the lag distances further apart than these ranges were statistically independent. Therefore, in Table 2, the ranges provide an estimate of areas of similarity. The results showed the presence of nugget effect for vc type, mating type and vc/mating genotype (Table 2). The nugget effect emphasizes that as distance goes to zero, the variograms do not approach zero variance. In order to evaluate the degree of spatial dependence, the ratio of the nugget (C_o) to the sill (C_o+C) was calculated for each variable. This ratio was 0.58 for vc type, 0.54 for mating type, 0.70 for vc/mating genotype. Since each ratio was >0.25 and <0.75, the variables showed moderate spatial dependence.

Discussion

Analysis of the population structure of *C. parasitica* in the Aydın Mountains based on vc and mating type traits indicated that *C. parasitica* has low genotypic diversity. Only two vc types (EU-1 and EU-12) and four vc/mating genotypes (*EU-1/MAT-1*, *EU-12/MAT-1*, *EU-1/MAT-2*, and *EU-12/MAT-2*) were detected. Lack of or low vc-type diversity has also been previously detected in other chestnut-growing regions of Turkey (Gürer et al. 2001; Çeliker and Onoğur 2001; Akıllı et al. 2009). In 2001, only one vc type, EU-1, was reported in the Marmara and Black Sea regions (Gürer et al. 2001; Çeliker and Onoğur 2001). However, in a recent study, a noteworthy increase in vc-type diversity was observed in the Black Sea Region (Akıllı et al. 2009). In that report, EU-1 remained the dominant vc type in all areas studied; however, EU-12 and several novel vc types for Turkey, such as EU-2, EU-5, and EU-14, were also recorded. Although perithecia of *C. parasitica* have been detected in the Black Sea region (personal observation in 2009), the role of sexual reproduction on the increase in vc-type diversity has not yet been clarified.

Similar to many other fungi, the population structure of *C. parasitica* is profoundly affected by its migration or dispersal mechanism and mating system (Milgroom and Peever 2003; Liu et al. 2000). Migration may increase gene diversity in local populations by introducing new individuals carrying novel alleles from other populations. The existence of four genotypes of *C. parasitica* in the Aydın Mountains indicates that there were at least four different introductions of the pathogen. However, it is

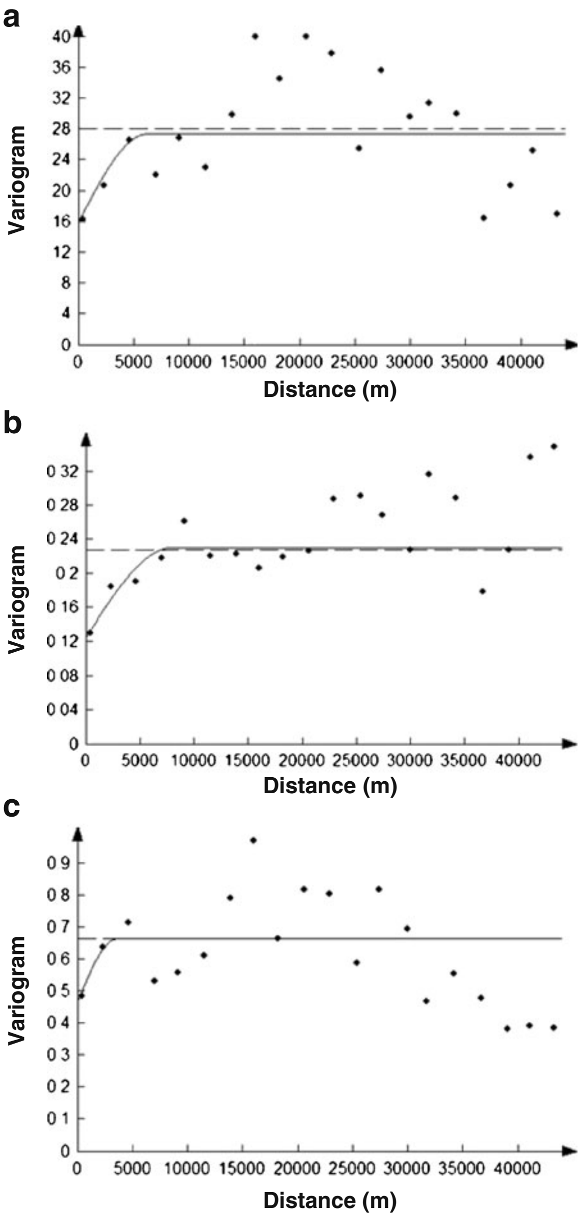

Fig. 5 Experimental variograms of *vc* types (**a**), mating types (**b**) and vc/mating genotypes (**c**) of *Cryphonectria parasitica* in the Aydın Mountains. The dotted lines represent experimental variograms, and the solid lines represent variogram models

difficult to make specific inferences about how the *C. parasitica* population in the Aydın Mountains is related to those reported in other parts of Turkey. The genotypes *EU-1/MAT-1* and *EU1/MAT-2* were first found in northern Turkey, in the Marmara and Black Sea regions, where *C. parasitica* was reported much earlier (Akdoğan and Erkman 1968; Gürer et al. 2001). Eventhough EU-12 has recently been reported from the Black Sea Region (Akıllı et al. 2009), a much wider distribution of EU-12 was previously reported from the Aegean Region (Çeliker and Onoğur 2001; Erincik et al. 2008). EU-12 is dominant in most Eastern European countries, including Greece and Bulgaria (Robin and Heiniger 2001; Perlerou and Diamandis 2006; Milgroom et al. 2008), which both share a border with Turkey. However, in order to make more accurate inferences about where *C. parasitica* now found in the Aydın Province migrated from, more complex genetic analysis of the population is necessary.

Mating systems of sexual populations may increase genotypic diversity as a result of the re-assortment of unlinked loci during sexual reproduction (Milgroom 1995; Cortesi and Milgroom 1998). Low vc-type diversity in the *C. parasitica* population in the Aydın Mountains may be an indicator of a lack of sexual reproduction. Sexually compatible genotypes of EU-1 and EU-12, such as *EU-12/MAT-1* vs *EU-1/MAT-2* and *EU-1/MAT-1* vs *EU-12/MAT-2*, were found in close proximity at several sampling spots; however, no vc types other than EU-1 and EU-12 were detected in these locations. Since EU-1 differs from EU-12 by four *vic* loci, 14 new vc types are expected to be generated from their sexual recombination (Cortesi and Milgroom 1998). These results suggest that no sexual crossing occurs yet between EU-1 and EU-12. In the Black Sea Region, two recently reported vc types (EU-2 and EU-14; Akıllı et al. 2009) are known to be two of 14 vc types, which could have been generated from the EU-1 and EU-12 cross (Cortesi and Milgroom 1998). High vc-type diversity of *C. parasitica* was observed in some European countries such as France, Italy, Switzerland, Bosnia-Herzegovina (Robin and Heiniger 2001) and Croatia (Krstin et al. 2008), where *C. parasitica* is known to reproduce sexually. On the other hand, certain Balkan countries such as Macedonia (Sotirovski et al. 2004) and Greece (Perlerou and Diamandis 2006), where *C. parasitica* did not have sexual stage, were reported to have lower vc-type diversity.

The presence of both *MAT-1* and *MAT-2* isolates in the same population is the first requirement for sexual reproduction in heterothallic *C. parasitica* populations (Marra and Milgroom 2001). Even though both mating types were detected in nearly all subpopulations in the Aydın Mountains, we found no evidence

Table 2 Theoretical variogram model parameters for observed data for vegetative compatibility (vc) types, mating types and vc/mating genotypes of *Cryphonectria parasitica* in the Aydın Mountains

Variable	Type	Nugget (C_o)	SV (C)	Sill (C_o+C)	C_o/(C_o+C)	Range (m)	Lag distance (m)		Goodness of fit
							Interval	Maximum	
vc type	Spherical	15.96	11.48	27.44	0.58	6160	2300	43600	0.13317
Mating type	Spherical	0.126	0.105	0.231	0.54	7920	2300	43600	0.04908
Genotypes (vc/mating)	Spherical	0.469	0.200	0.669	0.70	3520	2300	43600	0.12510

that *C. parasitica* is reproducing sexually. Mating type ratio data were apparently not reliable because the results varied depending on the spatial scale. For example, according to mating type data for the Aydın Province, the *MAT-1*:*MAT-2* ratio was not significantly skewed from 1:1. However, analysis of the data on a finer scale at the county level indicated that the ratios deviated significantly from 1:1 in nearly all subpopulations. Besides that, no perithecia were observed on natural cankers during sampling in this study, nor in the previously conducted studies in the region (Erincik et al. 2007). Similar to our results, both mating types were present in certain parts of Europe such as Macedonia (Sotirovski et al. 2004), Southern Italy (Milgroom et al. 2008) and North-West Spain (Montenegro et al. 2008), but no perithecial stage of *C. parasitica* was reported.

The spatial structure of the *C. parasitica* population, examined using geostatistical analysis, indicated that spatial autocorrelation existed among neighbouring *C. parasitica* isolates for vc types, mating types and vc/mating genotypes. This means that each type or genotype is patchy in its distribution within a specified distance. Those patches were evident in the probability occurrence maps generated by kriging. A critical factor related to the spatial structure of *C. parasitica* is whether *C. parasitica* disseminate by ascospores or by conidia (Milgroom et al. 1991; Liu et al. 2000). Dissemination of *C. parasitica* by rain-splash conidia was restricted within a short distance, thus resulting in a clonal population that exhibits positive spatial autocorrelation within small distances (Milgroom et al. 1991; Dutech et al. 2008). By contrast, sexual ascospores are genetically more diverse due to recombination and can be disseminated by wind over long distances (Milgroom and Lipari 1995; Liu et al. 2000), creating a population that shows a lack of or weak spatial autocorrelation. In our study, the restricted spread of vc types, mating types and vc/mating genotypes in the Aydın Mountains suggest that *C. parasitica* is disseminated by asexual inocula such as conidia and mycelia rather than by ascospores.

The pattern that was observed in the population structure of *C. parasitica* may be not only related to the dispersal mechanism of the inoculum but may be also influenced by habitat, human activities and regional topography (Hoegger et al. 2000). Our results indicated that each site in the Aydın Mountains has a different population structure. EU-1 (77%) was found to be dominant in the southern site, whereas EU-12 (70%) was dominant in the northern site. On the other hand, no *MAT-1* isolate was found in the eastern site. The Aydın Mountains have a very complex topography that is fractured into many small mountains and hills separated by dips and narrow valleys at various elevations. Therefore, these mountains and valleys may play a role as barriers inhibiting natural long-distance spread of *C. parasitica*. In Switzerland, it has been suggested that the Alps posed a barrier that inhibited the dissemination of wind-dispersed ascospores and vectors, separating clearly the northern *C. parasitica* population from the southern population (Hoegger et al. 2000). On the other hand, narrow continuous passageways or gates in the mountains may function as corridors, making connections between sites of the mountain and facilitating pathogen spread. For example, even though Sultanhisar County is located in the southern site, the population structure of *C. parasitica* in this county was very similar to that obtained from the northern site.

In the Aydın Mountains, because of the absence of natural hypovirulence (Erincik et al. 2008), the introduction of hypovirulent strains to blighted areas may be considered in the management of chestnut blight. Low vc-type diversity in the region will favor hypovirus transmission, which makes the population ideal for biological control. However, the results of this study indicate that the fungus has the potential for sexual reproduction in some areas where both mating types are present in close proximity to each other. The risky areas for the generation of new vc types due to sexual reproduction and the geographical distribution of each mating type were illustrated on the surface maps. These illustrations will be very useful for choosing a proper hypovirulent strain for the area in order to avoid bringing one mating type into contact with the other. They will be also used for monitoring changes in the population structure of *C. parasitica* in the future.

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