Genetic diversity among strains of *Sordaria fimicola* from contrasting environments

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Abstract. Genetic diversity was studied in populations of the soil fungus *Sordaria fimicola* at climate-contrasting environments using 132 AFLP markers. Six populations were tested from opposing slopes at 'Evolution Canyon' in Nahal Oren, Israel: three (upper, middle, and lower) from the harsher, drier south facing slope and the parallel three from the lusher, milder north facing slope. Analysis of molecular variance (AMOVA) showed that more genetic diversity among populations of *S. fimicola* was derived from between slopes than from within slopes; and F-statistics analysis suggested low estimations of inter-slope gene flow. In addition, clustering analysis (UPG MA) clearly grouped the six wild populations according to their appropriate slopes. These findings suggest that migration of *S. fimicola* between the two slopes of 'Evolution Canyon' is relatively small and some isolation barriers between opposing populations probably exist.

Key words: AFLP, ascomycetes, Evolution Canyon, Israel, Mediterranean

Introduction

During the last decade the use of DNA markers greatly contributed to the understanding of population genetic structure. Amplified Fragment Length Polymorphism (AFLP), a reliable powerful fingerprinting technique (reviewed by Mueller & Wolfenbarger 1999), has already been applied to several different fungi species (Majer *et al.* 1996; Rosendahl & Taylor 1997; Hogberg & Stenlid 1999; Boucias *et al.* 2000; Frisvad *et al.* 2005). In this study, AFLP technique was applied to populations of *Sordaria fimicola* (Roberge) Ces. et De Not. (Ascomycota) from 'Evolution Canyon', Israel.

'Evolution Canyon' is located at Lower Nahal Oren, Mt. Carmel, Israel (10 km south of Haifa). Its opposite slopes are separated by only 100 m at the bottom and 400 m at the top. However, the slopes represent great physical and biotic contrast and differ in microclimatic conditions (Pavlíček *et al.* 2003). The relatively mild north facing slope (NFS) mainly contains a Mediterranean-type evergreen maquis. The south facing slope (SFS) receives much higher solar radiation, and consists mainly of an open park forest and African-like savannah grassland. In these contrasting microclimate environments, a long term evolutionary and ecological research has been conducted (reviewed by Nevo 1997, 2001). Here, many unrelated organisms were tested as for their population structure. Genetic diversity among slopes was tested in populations of each of theses species, usually revealing different genetic structuring between inter-slopes populations (Nevo 1997, 2001; and see many references therein). In this across-phylogeny research, the representative of the fungi kingdom is *S. fimicola* (Lamb *et al.* 1998, 2000; Saleem *et al.* 2001), a common coprophilous micromycete (Olive 1974; Domsch *et al.* 1993). This fungus is distributed worldwide, in various different habitats, usually in various forest soils, but it is found also in dry habitats (Ranzoni 1968; Grishkan *et al.* 2003). The aim of

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this study was to test the genetic diversity among *S. fimicola* populations from the different slopes at 'Evolution Canyon' and to further contribute to the across-phylogeny studies in this long term research project.

Materials and Methods

Fungal material

Dung samples were collected at 'Evolution Canyon', Lower Nahal Oren, Israel. Strains of *Sordaria fimicola* isolated from dung (totalling 51 individual strains), were gathered from south facing slope and north facing slope at six stations (three on each slope) located at different altitude: 60 m, 90 m, and 120 m. Previously heated (30 min at 60 °C) dung pieces were put in Petri dishes with malt-extract agar and incubated at 25 °C for 7-8 days (Warcup & Baker 1963). Strains of *S. fimicola* were transferred to fresh agar medium and then were grown in liquid culture in Czapek Yeast medium for 10-14 days at 25° C.

AFLP analysis

DNA was extracted using DNAzol extraction kit (MRC Inc., USA). AFLP analysis was performed as described by Vos *et al.* (1995), modified for a non-radioactive method by using an automated sequencer, ALFexpress (Pharmacia, Sweden). Following a screening of 12 primer sets, three primer pairs were chosen for the analysis: *Mse*I+CCA/*Eco*RI+0, *Mse*I+CGC/*Eco*RI+0, and *Mse*I+GCT/*Eco*RI+0. These primer pairs were chosen as they were the clearest to score. In a reproducibility test, duplicates of six fungal strains were sampled: five of them were identical in all bands and one differed in a single band (99.2 % similarity). Product sizes (bands) were evaluated by ALFwin Fragment Analysis Software using a 50-500bp ladder as an external marker. In all primer combinations, bands were scored at the clearest range of 60-340bp.

Statistical analysis

Bands were scored as present or absent. The resulted data matrix was analysed using POPGENE software (Yeh *et al.* 1999) for population genetic diversity, genetic distances, F-statistics, estimating gene flow, and for obtaining an UPGMA clustering dendogram (based on Nei 1978). AMOVA 1.55 statistical program (based on Excoffier *et al.* 1992) was used for one-level and two-level analyses of molecular variance.

Results

The three different primer combination revealed a total of 132 AFLP markers, of these 115 (87.12 %) were polymorphic. Each of the 51 strains tested was found to be unique in its genotype. Genetic diversity values of *Sordaria fimicola* population tested were relatively high (H = 0.23-0.29, Table 1), with the exception of the population from the upper site at the north facing slope (H = 0.11). This is probably because of the relative scarcity of *S. fimicola* in this upper NFS site, as this site offers quite different conditions (hard rocks, cliffs, less shady) than those in the otherwise lusher NFS. Inter-slope genetic diversity showed that the total populations diversity in *S. fimicola* is higher on the SFS: by analyzing population diversity into two groups (NFS and SFS), the NFS has a lower mean genetic variability (H=0.30, Table 1) than the SFS (H = 0.33), but this difference is mainly due to the low value (H = 0.11) of the population at the upper NFS. The percentage of polymorphic loci was also higher in the SFS (78.03 %, Table 1) compared to the NFS (68.94 %).

Table 1. Genetic diversity of six populations of *Sordaria fimicola* from two opposing slopes of 'Evolution Canyon'

		N [a]	H [b]	P [c]	
By population					
South facing slope	Upper	8	0.27 (0.30)	48.48	ns
	Middle	9	0.29 (0.28)	57.58	< 0.05
	Lower	9	0.24 (0.28)	46.97	ns
North facing slope	Lower	9	0.28 (0.27)	52.27	ns
	Middle	9	0.23 (0.29)	43.94	ns
	Upper	7	0.11 (0.23)	20.45	< 0.005
By slope					
South facing slope	(SFS)	26	0.33 (0.24)	78.03	ns
North facing slope	(NFS)	25	0.30 (0.26)	68.94	ns

[a] N = sample size

[b] H = genetic diversity (Nei 1973, standard deviations are in parentheses)

[c] P = percentage of polymorphic loci; ns = non significant (χ^2 test, p = 0.05)

Analysis of molecular variance (AMOVA) suggested that more genetic diversity in *S. fimicola* derived from between slopes than from within slopes. Two-level variance partitioning show that among slope variance components contributed 8.47 % of the total diversity, a higher value than the 5.35 % which was contributed by the among-populations variance components (Table 2). In addition, an UPGMA dendogram (Fig. 1) clearly grouped the six wild populations into two groups according to their appropriate slopes (while the upper NFS population is an outlier to all five populations, as it is genetically more distant, see Table 3). Genetic distances analysis (Table 3) suggests that identity is usually higher between populations of the same slope, probably because of the slope effect: dung from the higher sites could easily travel down the slope with strains of *S. fimicola* colonizing it. Moreover, *F*-statistics analysis and *Nm* estimations revealed that inter-slope gene flow is relatively small: *Fst* values were 0.136 (for the NFS) and 0.199 (SFS); and *Nm* estimations were 1.59 (NFS) and 1.01 (SFS). These estimations suggest that populations on the two slopes are principally isolated from one another while the migration of a small number (1 or 2) of migrants per generation could occur.

Table 2. Analysis of molecular variance AMOVA of 51 *Sordaria fimicola* strains in six populations grouped into two opposing slopes

Source of variations	df[a]	Sum of squares	Mean sum of squares	Variance components	Total (%)
Two-level for six populations					
among slopes	1	41.30	41.30	1.35	8.47[b]
among populations	4	92.68	23.17	0.86	5.35[b]
among strains	45	620.55	13.79	13.79	86.17[b]
One-level for two slopes					
among slopes	1	41.30	41.30	1.61	10.03[b]
among strains	49	705.11	14.39	14.39	89.97[b]

[a]df = degrees of freedom

[b]$p < 0.001$ at variance components significance after 1000 permutations

Table 3. Genetic distances between the six populations of *Sordaria fimicola* tested from the two slopes of 'Evolution Canyon'

		South Facing Slope			North Facing Slope	
		Upper	Middle	Lower	Lower	Middle
NFS	Upper	0.098	0.101	0.097	0.125	0.109
	Middle	0.092	0.103	0.118	0.066	
	Lower	0.115	0.064	0.086		
SFS	Lower	0.074	0.036			
	Middle	0.079				

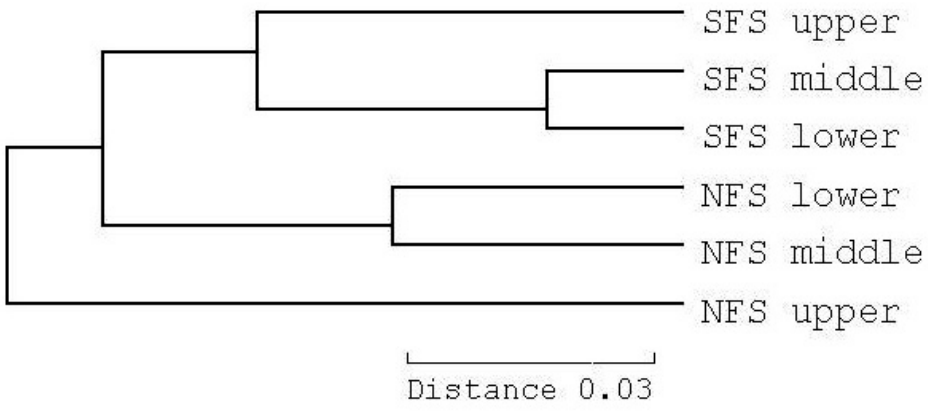

Fig. 1. Dendogram of six populations of *Sordaria fimicola* from two opposing slopes of 'Evolution Canyon. SFS = south facing slope, NFS = north facing slope

Discussion

In the light of these results, one can estimate the relative importance of some evolutionary forces (e.g., migration, drift and genome rearrangement) acting on *S. fimicola* populations in 'Evolution Canyon':

(i) Theoretically, inter-slope migration followed by gene flow could occur in *S. fimicola* with the help of grazing animals which can move between slopes. However, it seems that this is not the case here and inter-slope migration is negligible. Lamb *et al.* (2000) found that some SFS strains of *S. fimicola* are cross-incompatible with NFS strains, suggesting some genetic barriers between inter-slope populations. In the broader prospect of 'Evolution Canyon', similar findings on the lack of inter-slope migration were reported in a cyanobacterium (Krugman *et al.* 2001), in barley (Owuor *et al.* 1997), and even in moving organisms like rodents (Blaustein *et al.* 1996) and flies (Korol *et al.* 2000). Summing up the *F*-statistics analysis and *Nm* estimations presented here and the previous reports on cross-incompatibility (Lamb *et al.* 2000), it is suggested that in *S. fimicola* gene flow between slopes is very little.

(ii) The general patterns of slope differentiation at 'Evolution Canyon' in each of dozens of various species tested here suggest that it could hardly come out by chance alone (Nevo 1997, 2001). In this study as well (without assuming whether the anonymous AFLP markers are subject to selection or neutral), it is speculated that genetic drift or other stochastic forces are probably not (or nearly not) involved in the separation of between-slopes populations.

(iii) Mutation and recombination are two evolutionary forces which were already studied in *S. fimicola* populations from 'Evolution Canyon': differences between strains from opposing slopes were manifested both in mutation rate (Lamb *et al.* 1998) and in crossing-over and gene conversion rate (Saleem *et al.* 2001). In both cases the rate was higher on the SFS. Generally speaking, the SFS in 'Evolution Canyon' is a harsher environment than the opposing NFS. It receives higher solar radiation than the NFS (Pavlíček *et al.* 2003). Thus, the SFS is warmer, drier, climatically more fluctuating and less predictable than the NFS (Nevo 1997). Theoretically, higher genomic change rates are therefore expected at the SFS (Felsenstein 1976; Zhuchenko *et al.* 1985; Korol *et al.* 1994). In that case, genomic changes and genetic diversity could be promoted by environmental factors and therefore could be stronger in the SFS (Nevo 1997). Then, it is possible that selection will maintain this accumulated genetic diversity (Hedrick 1986; Nevo 2001).

The genetic evidence presented in *Sordaria fimicola,* together with the microscale-model of 'Evolution Canyon' may well be a challenging ground for further studies of this fungus, attempting at a comprehensive understanding of local evolutionary forces.

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