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The mode of reproduction in natural populations of ascomycetous fungus, *Emericella nidulans*, from Israel

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Summary

The mode of reproduction of the soil ascomycetous fungus *Emericella nidulans* of Israeli populations was studied using 15 microsatellite (simple sequence repeats or SSR) trinucleotide markers. The study was performed in three canyons: two located in the northern part of Israel (Mount Carmel and western Upper Galilee) and one in the southern Negev desert. In each canyon, *E. nidulans* strains were isolated from the opposite slopes and (in the desert canyon) the valley bottom. Testing the reproductive structure of the populations indicated the presence of sexuality in the northern population and predominant clonality in the desert population. The predominantly clonal character of the desert population of *E. nidulans* was explained by the assumption that for relevant multilocus systems of a fungus, only several haplotypes can survive in the rather constant, extremely stressful desert conditions. Additionally, the very low density of *E. nidulans* populations in the soil of the desert canyon, which reduces the probability of finding a sexual partner, might favour predominant clonality via selfing. Increasing sexuality in *E. nidulans* populations on the north-facing slopes of the northern canyons may be a result of biotic stress (pressure of competitive fungal species), due to the more mild ecological conditions in these canyons.

1. Introduction

Evolutionary and ecologically important aspects of variability and adaptation of fungi are highly associated with their mode of reproduction. Fungi are known to display diverse reproduction patterns (Taylor *et al.*, 1999; Dyer, 2008). There are two major kinds of reproductive patterns that differ in principle: sexual (by means of meiospores) and asexual or clonal (by means of mitospores). These reproductive modes perform different functions in the life cycle of a fungus and are considered to have their own advantages and disadvantages as evolutionary strategies. Sex is regarded as an expensive process because the amount of offspring for one sexual parent is less than for one clonal parent, although for fungi, the actual cost of sex is much lower compared to animals and plants (e.g. Aanen & Hoekstra, 2007). It is postulated that sexual reproduction serves as a 'conservative' mechanism preserving the genome from degradation by facilitating selection against harmful mutations, and as a factor of genome flexibility that increases the probability of survival in a competitive and/or changing environment and expedites the appearance of evolutionary innovations (Maynard Smith, 1978; Elliot, 1994; Korol *et al.*, 1994; Otto & Gerstein, 2006; Goddard, 2007).

On the other hand, asexual propagation is believed to be a successful evolutionary strategy for well-adapted genotypes in a stable, even if extreme, environment (Murtagh *et al.*, 2000). About 20% of known fungal species are considered to be asexual (Dyer & Paoletti, 2005). For clonal fungi, there is a potential for mitotic recombination in heterokaryons via a parasexual cycle, which may be an important mechanism for diversification within a taxon (e.g. Debetes, 1998). Recent studies have shown that some morphologically asexual parasitic and saprotrophic species in fact possess a sexual cycle (Braumann *et al.*, 2008, and references therein). The sexual phase may

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be either cryptic or rare, occurring only under very special conditions hardly observable in laboratory cultures. One such case is *Aspergillus fumigatus*, for which a fully functional sexual reproduction cycle leading to the production of heterothallic teleomorph *Neosartorya fumigata* was recently discovered (O'Gorman *et al*., 2009). Thus, for some fungal species, the sexual states may have been overlooked for a long period of time. Due to the diversity of reproductive strategies, fungal species represent an ideal target for experimental studies of the evolution of sex, to fill the gap between the ever-increasing spectrum of theoretical models and scenarios along with very limited empirical evidence for nearly every important characteristic on which these models are based.

The ascomycete *Emericella* (*Aspergillus*) *nidulans* (Eidam) Vuill. is a fungus that in culture easily generates two morphologically distinct kinds of sporulation: sexual (teleomorphic state, producing ascospores) and asexual (anamorphic *Aspergillus* state, producing conidia). Both spore types can be produced via clonal (selfing for ascospores and asexual cycle for conidia) and recombinant (outcrossing for ascospores and parasexual cycle for conidia) modes of reproduction (Pontecorvo, 1956). Historically, *E. nidulans* was the first fungus for which the parasexual cycle was described (Pontecorvo *et al*., 1953). The fungus is homothallic (i.e. self-compatible or self-fertile) and, as a rule, haploid, but it can also exist as a heterokaryon and as a diploid. *E. nidulans* is widely distributed in different climatic and geographic areas across the world (Domsch *et al*., 2007), including Israel (Volz *et al*., 2001).

Previously, we studied the genetic variation of Israeli populations of *E. nidulans* on regional and local scales using seven microsatellite (simple sequence repeats or SSR) markers (Hosid *et al*., 2008). The study was performed in the framework of the 'Evolution Canyon' research programme at the Institute of Evolution, University of Haifa, focusing on the effect of microscale environmental variability on biodiversity patterns (Nevo, 2001) in three 'Evolution Canyons' (ECs). The first two canyons (EC I and EC II) are located in the northern part of Israel at a distance of 38 km from each other. The desert EC III is located southward at a distance of nearly 350 km from the northern ECs. In each canyon, *E. nidulans* strains were isolated from the opposite slopes (in EC III also from the valley bottom (VB)). All three EC populations of *E. nidulans* were found to be genetically distinct from one another. The estimated genetic differentiation corresponds to geographical and ecological differences among the three microsites. On a regional scale, SSR polymorphism tended to increase with the severity of ecological conditions.

Our present study, based on 15 microsatellite markers, focuses on testing the reproductive, sexual versus clonal structure of the aforementioned *E. nidulans* populations. The population reproductive structure can be assessed by measuring correlated genetic diversity displayed by alleles at different loci and by comparison of phylogenetic trees constructed using molecular variation at each locus (reviewed in Taylor *et al*., 1999). In this paper, we demonstrate that the reproductive mode in *E. nidulans* may be population-specific: occurrence of sexuality was found in the northern populations and predominant clonality was found in the desert population.

2. Material and methods

(i) *Sites of study*

The study was conducted in three ECs: Lower Nahal Oren, Mt. Carmel (EC I, 32°43′N, 34°58′E); Lower Nahal Keziv, western Upper Galilee (EC II, 33°02′N, 35°11′E) and Nahal Shaharut, the southern Negev desert (EC III, 29°55′N, 34°58′E). In each canyon, samples were taken from the two opposite slopes – north-facing slope (NFS) and south-facing slope (SFS), separated by 50–150 m at the VB. EC I and EC II, located in the northern part of Israel, are characterized by sharply different microclimatic conditions (Pavlicek *et al*., 2003). Different plant communities have developed on the opposite slopes: garrigue or savannoid, open park forest on the south-facing 'African' slopes and dense forest on the north-facing 'European' slopes. EC III represents an extreme desert location, with very sparse shrub vegetation growing only on the NFS and VB. For a detailed description of the canyons, see Hosid *et al*. (2008).

(ii) *Soil sampling and isolation of strains*

Soil samples were taken from the upper layer (1–3 cm deep) of the SFS and NFS at EC I and EC II, and from the NFS and VB at EC III. Altogether, 175 strains of *E. nidulans* were isolated: 43 from EC I (18 and 25 from the SFS and NFS, respectively, in 2002), 55 from EC II (29 and 26 from the SFS and NFS, respectively, also in 2002) and 77 from EC III (61 from the VB, 16 from the NFS, in 2004). The strains were isolated by the soil dilution plate method (Davet & Rouxel, 2000) using dilutions of 1 : 10, 1 : 100 and 1 : 1000 for soil : sterile water. The strains were identified using cultural and morphological criteria (Klich, 2002). The isolated strains are preserved at 4 °C in the culture collection of the Institute of Evolution, University of Haifa.

Growth of the strains, DNA isolation, PCR amplification and statistical analysis were performed as it was described in our previous work (Hosid *et al*., 2005, 2008). The Spearman rank correlation test

Table 1. *Description of SSR markers of* E. nidulans

Locus	Primer sequence (5′–3′)	Repeat	Linkage group	Contig	Product size (bp)	*D* (kb)	*D* (% of chrom. arm)
NC1L3	CGACGACGAGGATAAGGAAG TGATTGTCGGGGCTTAATTC	$(GAAGAC)_{20}$	I	1·11	248	269·7	18·3
NC1L5	TACGACCGTCATTGTTGCAT TGAATGGATTGCAGGATCAA	$(AGG)_{8}$	I	1·11	227	1513·4	69·1
NC6L3	ACACTGGCGACCGAGAAAC CAGATCTCCGCCAGTTTGAT	$(TCCTGC)_{11}$	VI	1·51	295	1075·2	38·8
NC6L4	GATTGAGCAGGACGAGAACC CAGCAAACATTCTGAAGAAGCA	$(TGAGCC)_{14}$	VI	1·51	180	1328·7	47·9
NC1L2	TGCTTCTGTCGTTGTGGGCTAC AAGACGGGAGATGGAATGTGTTTG	$(CCT)_{19}$	I	1·10	165	1010·5	46·1
NC6L2	CGGTTGATGTTGGTTGTCCTG ACCGCAATCGCAGAACAAGAG	$(TGA)_{32}$	VI	1·49	228	2296·6	82·8
NC8L6	GAAGAATCTGAGGGCGTCAC GCGTCACAGACGCAGATTT	$(CAACAG)_{15}$	VIII	1·14	240	1512·6	35·3
NC8L2	CGTTGGCTGTGGTTACGGAC TGGACTGATTGCCGGGTTAATC	$(CTT)_{7}$	VIII	1·10	178	2111·1	52·1
NC8L4	ACTCCGCAACAGTTCGCTCAG TCTGAGCCTGGTATGTCTGGG	$(CAG)_{12}(CAA)_{11}$	VIII	1·14	185	1268·1	36·6
NC8L1	TCAGAGGATCCAGGACGACTAG GACCTGTGTCACCTACGACTGC	$(GCA)_{10}$	VIII	1·10	154	2111·1	52·1
NC8L5	ACCTATGCGCTTTGTTTCTGACT CCAAGTAGTGAGGCAATGGG	$(CAA)_{12}$	VIII	1·16	284	1268·1	15·1
NC2L1	CAGGAGTTGGCGACATCGTCTG GTTTCGGTCCCTGGTTTCTGTGTC	$(GCA)_{25}$	II	1·61	321	1559·3	85·7
NC6L1	CCATCACCATCCGTACCTCAC AAGCTCCACAGCCGCATTAC	$(CCT)_{14}(CCA)_{17}$	VI	1·49	291	2288·7	78·4
NC1L1	GCTGGCGACGATGATCCTAC CAGATCATGAACACGAGCAACC	$(GGN)_{33}$	I	1·11	285	1915·5	87·5

(StatSoft, 1996) was employed to estimate correlation between the variability of SSR markers (variance in repeat number) and physical distance from the markers to the centromere (% of chromosome arm length) (Table 1).

(iii) *Mode of reproduction of* E. nidulans *populations*

Mode of reproduction of *E. nidulans* populations was tested using two methods for mutual control of the results obtained: the index of association (IA) and linkage disequilibrium (LD).

(1) The IA was calculated using the MultiLoc program (http://www-bs.informatik.uni-tuebingen.de/Services/MultiLoc). This index quantifies the amount of recombination among a set of sequences and detects association between alleles at different loci (Maynard Smith *et al*., 1993). In populations with frequent recombination events, the value of IA is expected to be zero. Clonal populations are identified by an IA value that differs significantly from zero.
(2) LD was estimated by the χ^2 using the *Arlequin* program (Excoffier *et al*., 2005). With sexual reproduction, low LD values are expected for the vast majority of tested marker pairs. Moreover, pair-wise LDs should negatively correlate with physical distances between loci. To estimate the correlation between LD and physical distances for SSR marker pairs, LD was expressed as a negative logarithm of the *P*-value for the corresponding χ^2 statistic. As with the IA-based test, a non-significant LD indicates sexuality in the tested populations.

3. Results

A slight tendency for decreasing LD with increasing distance between SSR loci was found in the EC I population ($P<0{\cdot}03$) and the subpopulations of $NFS_{EC\ I}$ ($P=0{\cdot}11$) and $NFS_{EC\ II}$ ($P=0{\cdot}25$). This pattern, together with the relatively low level of LD, may point to clonal reproduction mixed with sex and recombination. In the EC III population with more marker pairs used, a significant correlation between LD and physical distances between the markers was obtained for the NFS subpopulation (Fig. 1). In the EC III population, particularly in its VB subpopulation, we can also see a tendency, although non-significant, for decreasing LD with growing physical

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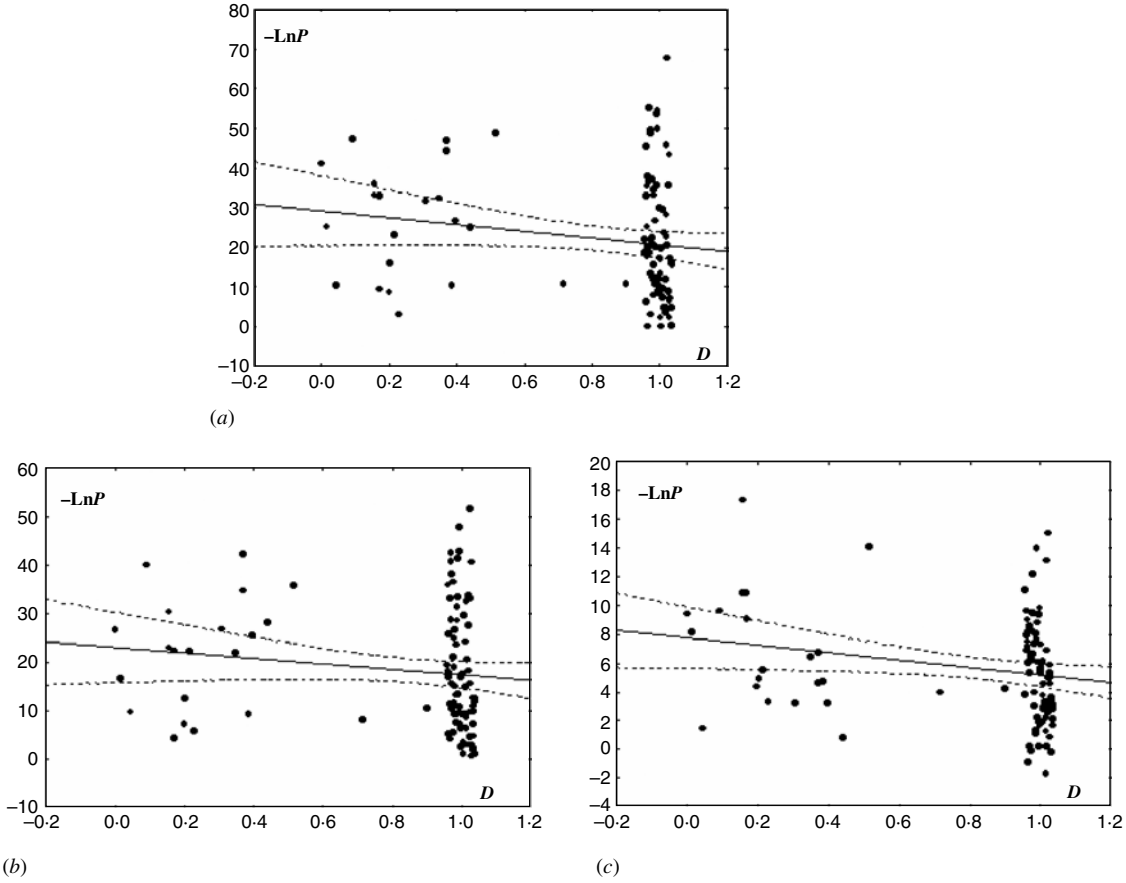

Fig. 1. Correlation between LD and physical distance (D) between pairs of tested loci in the populations and subpopulations of *E. nidulans* from EC III. Distance D was taken as equal to 1 for loci from non-homologous chromosomes (for obvious reasons, $1+\varepsilon$ was used instead of 1, with ε uniformly distributed in $[-0{\cdot}05, 0{\cdot}05]$) and equal to the proportion of distance between loci to chromosome length (in bp) for loci from the same chromosome. LD was scored as $-$Ln of P-value in the χ^2-test. The graphs show the scored correlation between Y and X with 95% confidence (StatSoft 1996). (*a*) For EC III, $R=-0{\cdot}17$, $P=0{\cdot}09$, $-\mathrm{Ln}P=29{\cdot}24–8{\cdot}49*D$; (*b*) for EC III (VB), $R=-0{\cdot}14$, $P=0{\cdot}17$, $-\mathrm{Ln}P=23{\cdot}01–5{\cdot}58*D$; (*c*) EC III (NFS), $R=-0{\cdot}21$, $P=0{\cdot}04$, $-\mathrm{Ln}P=7{\cdot}78–2{\cdot}58*D$.

distance between SSR loci. This tendency suggests that recombination may be present in this population. No significant correlation between LD and physical distance was found for the EC II populations, indicating the presence of clonal propagation in this population.

The results on LD (χ^2-test) and IA for the *E. nidulans* populations are shown in Table 2. For the EC I and EC II populations, only 13% and 33% of the χ^2 values from the pairwise comparisons, respectively, were significant. In contrast to EC I and EC II, for LD in the EC III population χ^2 values were significant for 99% of marker pairs; a similar tendency was observed for IA. The obtained results point to the presence of sexuality in the EC I and EC II populations and predominant clonality of the EC III population.

A significant positive correlation was obtained between the variance in the marker repeat number and physical distance from the markers to the centromere ($R=0{\cdot}314$, $P=0{\cdot}009$). Such a correlation can serve as additional evidence of the recombination presence in the tested populations. Similar to many other organisms, in *E. nidulans* the proximity to the centromere is known to greatly reduce the rate of recombination, a phenomenon referred to as the 'centromeric effect' (Korol *et al.*, 1994; Aleksenko *et al.*, 2001; Espeso *et al.*, 2005).

According to all estimated characteristics and additional tests (scoring of the correlation between inter-strain digital distances for each pair of loci in the populations and the bootstrap consensus analysis – data not shown), we consider the EC I and EC II populations to be predominantly sexual, in contrast to the EC III population, which showed a predominantly clonal structure.

4. Discussion

For multilocus marker systems, scoring IA and measuring LD by χ^2-test has been traditionally used in examining whether a sexual or clonal mode of

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Table 2. *LD* (χ^2*-test, below the diagonal*) *and IA* (*above the diagonal*) *for the populations of* E. nidulans *from EC I* (*a*), *EC II* (*b*) *and EC III* (*c*)

(a) χ^2/IA	NC8L4	NC8L2	NC8L1	NC8L5	NC8L6	NC6L1	NC6L4	NC1L1	NC1L2	NC2L1
NC8L4	–	−0·03	0·04	0·01	0·07	−0·1	0·25	0·03	−0·1	0·03
NC8L2	39·65	–	0·07	−0·02	0·004	−0·07	−0·13	0·08	0·11	0·08
NC8L1	**64·55**	**70·55**	–	−0·04	−0·14	0·05	0·003	−0·03	−0·001	−0·04
NC8L5	28·66	23·88	30·35	–	0·04	0·05	0·02	0·04	−0·05	−0·001
NC8L6	48·36	51·56	86·69	**54·20**	–	−0·1	−0·02	0·03	−0·003	**−0·03****
NC6L1	8·45	12·19	22·95	9·76	13·07	–	0·04	0·04	−0·12	0·12
NC6L4	**55·12**	43·14	72·72	34·89	66·44	16·55	–	0·01	−0·15	**−0·08****
NC1L1	9·14	14·90	28·46	11·31	18·57	10·43	20·11	–	−0·13	−0·03
NC1L2	30·37	**44·03**	66·62	20·27	56·55	15·24	46·95	15·88	–	**−0·1****
NC2L1	44·50	51·27	87·55	40·16	**82·06**	27·76	50·26	14·01	61·12	–

(b) χ^2/IA	NC8L4	NC8L2	NC8L1	NC8L5	NC8L6	NC6L1	NC6L4	NC1L1	NC1L2	NC2L1
NC8L4	–	−0·08	0·02	−0·006	−0·01	−0·03	−0·02	0·05	0·03	0·01
NC8L2	**58·29**	–	−0·04	−0·07	−0·05	0·02	−0·05	0·11	−0·05	0·14
NC8L1	29·12	21·51	–	0·07	0·02	0·08	0·01	0·03	0·03	0·04
NC8L5	22·20	**38·81**	22·73	–	−0·14	−0·1	−0·03	−0·05	−0·004	0·04
NC8L6	45·79	**58·94**	18·18	**46·45**	–	0·09	−0·003	0·05	−0·01	−0·21
NC6L1	30·49	17·08	**31·46**	18·51	35·32	–	0·11	−0·004	0·03	0·05
NC6L4	46·57	**65·23**	25·93	30·51	61·68	28·46	–	0·11	0·12	0·02
NC1L1	26·10	24·38	**18·40**	8·91	15·05	11·04	12·91	–	0·06	0·07
NC1L2	49·64	55·51	25·27	31·66	**79·25**	27·87	54·99	27·22	–	0·22
NC2L1	59·60	**76·37**	28·98	**47·20**	**63·86**	**40·63**	**66·12**	**39·17**	**75·19**	–

(c) χ^2/I_A	NC8L4	NC8L2	NC8L1	NC8L5	NC8L6	NC6L1	NC6L2	NC6L3	NC6L4	NC1L1	NC1L3	NC1L5	NC1L2	NC2L1	NC3L2
NC8L4	–	**0·27***	**0·16***	**0·24***	**0·07***	**0·17***	**−0·01***	**0·16***	**0·24***	**0·18***	**0·28***	**0·13***	**0·27***	**−0·01***	**−0·05***
NC8L2	**119·72**	–	**0·25***	**0·64***	**0·11***	**0·36***	**0·14***	**0·33***	**0·46***	**0·15***	**0·55***	**0·07***	**0·63***	**0·02***	**−0·06***
NC8L1	**180·96**	**107·05**	–	0·25[s]	0·15	0·15	−0·002	0·11	0·27	0·13	0·28	−0·01	0·28	0·02	−0·02
NC8L5	**150·10**	**116·79**	**153·54**	–	**0·13***	**0·31***	**0·06***	**0·42***	**0·61***	**0·04***	**0·69***	**−0·003***	**0·72***	**−0·01***	**0·02***
NC8L6	**302·01**	**91·91**	**232·87**	**181·13**	–	**0·09***	**−0·02***	**0·12***	**0·12***	**0·03***	**0·18***	**0·07***	**0·03***	**0·04***	**0·11***
NC6L1	**181·19**	**121·89**	**138·31**	**149·26**	**213·12**	–	0·16	0·10	0·28	0·06	0·35	0·04	0·37	0·04	0·006
NC6L2	**130·87**	**61·20**	**75·03**	**92·50**	**138·14**	**91·23**	–	**0·01***	**0·05***	**0·11***	**0·05***	**0·14***	**0·17***	**0·14***	**−0·03***
NC6L3	**163·42**	**112·33**	**113·99**	**143·25**	**173·08**	**117·67**	**83·66**	–	**0·30***	**0·18***	**0·37***	**−0·05***	**0·47***	**−0·02***	**0·07***
NC6L4	**174·26**	**100·06**	**104·22**	**136·73**	**185·30**	**160·56**	**118·27**	**132·05**	–	**0·007****	**0·54****	**0·03****	**0·54****	**0·08****	**−0·07****
NC1L1	**168·96**	**107·37**	**145·87**	**135·11**	**222·72**	**172·11**	**90·22**	**142·51**	**122·18**	–	**0·04****	**0·08****	**0·02****	**0·02****	**−0·07****
NC1L3	**180·25**	**120·64**	**134·13**	**172·23**	**170·66**	**156·14**	**100·55**	**129·39**	**136·31**	**128·98**	–	**−0·005***	**0·70***	**0·04***	**0·03***
NC1L5	**220·79**	**85·97**	**133·96**	**129·33**	**311·76**	**160·81**	**88·41**	**129·18**	**113·25**	**176·88**	**129·84**	–	**0·08****	**−0·03****	**0·07****
NC1L2	**155·18**	**119·41**	**131·44**	**164·10**	**148·67**	**141·55**	**72·83**	**165·72**	**151·51**	**128·02**	**161·33**	**121·41**	–	−0·02	0·02
NC2L1	**167·96**	**67·00**	**149·48**	**84·12**	**266·56**	**138·33**	**107·72**	**92·05**	**118·33**	**168·27**	**92·74**	**202·56**	**95·67**	–	0·04
NC3L2	**172·16**	**85·38**	**96·03**	**99·47**	**238·16**	**125·17**	**64·48**	**97·58**	**115·54**	**145·07**	**112·15**	**132·45**	**110·84**	**171·27**	–

*$P<0·05$ and **$P<0·005$. Significant values of χ^2 are bolded.

propagation prevails in natural, including fungal, populations (Maynard Smith *et al*., 1993; Haubold *et al*., 1998; Walser *et al*., 2004; Tuthill, 2004; Stukenbrock & Rosendahl, 2005). The correlation between LD and physical distances was recently used for studying the reproductive structure of fungal populations (e.g. Tsai *et al*., 2008) in spite of some opposite data showing that a negative correlation between polymorphism and DNA distance did not prove the presence of recombination and could probably be caused by different independent mutations (Hey, 2000). In our study, both the IA and LD criteria as well as other methods (including correlation between genetic diversity indices and distance of SSR markers to the centromere) showed similar results. In the northern ECs, sexual propagation seems to contribute significantly to the *E. nidulans'* population structure, corroborating the findings on British populations of this fungus where recombination was shown to occur rather frequently (Geiser *et al*., 1994). A different pattern was displayed by yeast *Saccharomyces cerevisiae* from the northern EC I, where the population proved to be heterogeneous for ploidy level, with diploids showing sexual structure while tri- and tetraploids were predominantly clonal (Katz Ezov *et al*., 2006).

All tests employed indicated different predominant types of reproduction in the northern and desert populations of *E. nidulans*. Predominant clonality was found in the EC III populations inhabiting the extreme desert environment. This finding seems to contradict the conjecture that the sexual stage prevails in stressful conditions (e.g. Elliot, 1994) and our previous results on the relationship between ecological stress and reproduction mode in soil microfungi (Grishkan *et al*., 2003*a*). In that study, we showed a highly significant increase in the proportion of morphologically sexual species in mycobiota with an increasing salinity/aridity stress southwards in Israel. The question is whether this is really a contradiction. The presence of a morphologically expressed sexual stage in a homotallic fungus does not necessarily mean recombination occurs at the level of its population structure because of the ability of selfing. For example, *Eupenicillium* sp. with a known sexual stage in its life cycle was found to be predominantly clonal by molecular methods (Tuthill, 2004). All of our desert isolates easily produced a selfing sexual stage under laboratory conditions. Presumably, ascospores may be produced in natural desert *E. nidulans* populations.

An environment-based explanation of the predominantly clonal character of the desert population of *E. nidulans* may be associated with the assumption that for relevant multilocus systems of a fungus, only several gene combinations (haplotypes) can exist under extremely stressful conditions (such as high solar and UV radiation, very low moisture and organic matter content, see Grishkan *et al*., 2007). Our results imply that in the most extreme, but stable, conditions asexual propagation might be preferential, corroborating several other studies (e.g. Murtagh *et al*., 2000). For *E. nidulans* grown under laboratory conditions, carbon deficiency, light exposure and high salinity were shown to preferentially stimulate asexual reproduction of the fungus, while less edaphically stressful conditions favoured sexual development (Kap-Hoon *et al*., 2003). Additionally, very low density of *E. nidulans* in the soil of the desert EC, which reduces the probability of finding a sexual partner, might favour predominant clonality via selfing. A similar tendency was found in marginal plant populations (e.g. Levin, 1975; Nasrallah *et al*., 2004) and pioneer lichens (Murtagh *et al*., 2000), where selfing prevailed.

Many fungal populations seem to utilize mixed reproductive strategies (reviewed in Milgroom, 1996) with different proportions of sexuality and clonality. Recently, some evidence of the sexuality in species with unknown sexual stages was reported, e.g., in *A. fumigatus* (Dyer & Paoletti, 2005), *Aspergillus niger* and *Penicillium chrysogenum* (Braumann *et al*., 2008). Our results cannot reject the hypothesis that increasing sexuality in fungi relates to increasing stress severity. Such a tendency may be a general rule, but becomes invalid at too extreme stresses (at the 'edge of life'). Under such conditions, low population density (hence, the availability of a sexual partner) makes reproductive assurance a more important problem than the generation of recombination-dependent variation.

Another aspect of the problem is related to the interpretation of environmental severity. In addition to abiotic stress, one should consider the biotic components of the habitat. Competition stress (pressure of competitive fungal species) increased towards more mild ecological conditions in northern ECs (Grishkan *et al*., 2000, 2003*b*, 2007). Biotic stress is no less important to the evolution of sexuality than abiotic stress, as expressed in the Red Queen hypothesis of sex evolution (Hamilton *et al*., 1990; Korol *et al*., 1994; Gandon & Otto, 2007). This can explain the observed tendency of increasing sexuality in *E. nidulans* towards increasing biotic stress in the NFS populations at the northern ECs.

Our results demonstrate the suitability of fungi for testing the effect of environmental stress on the reproduction mode when conducted in the context of natural populations inhabiting contrasting ecological conditions. Further studies are needed to discriminate between the two main explanations of the observed difference in reproductive strategies of *E. nidulans* in the inspected populations, i.e., reproductive assurance and biotic stress.

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