Adaptive growth rates of fungi from *Aspergillus niger* group in contrasting environments: the Dead Sea and "Evolution Canyon" I (Israel) under different osmostress

Katerina Selezska, Leonid Brodsky & Eviatar Nevo*

Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31905, Israel

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Abstract. Filamentous fungi from *Aspergillus niger* group were isolated from the hypersaline Dead Sea water and the Mediterranean "Evolution Canyon" I, lower Nahal Oren, Mount Carmel. A comparison of growth rates of the strains collected from the Dead Sea and the "European" north- and "African" south-facing slopes of "Evolution Canyon" I, over a range of water activities, was provided. Media adjustments were made with different volumes of Dead Sea water. Strains from all habitats showed optimal growth rates at 5 % of Dead Sea water (a_w 0.983) and ceased growth at 65 % of Dead Sea water (0.785 a_w). However, significant interpopulation growth differences were detected (by the non-parametric Kolmogorov-Smirnov test) at different a_w. Under low salinities (< 15 %), "Evolution Canyon" I strains significantly differ from Dead Sea water strains in distributions of growth rates. Under high salinities (> 40 %), there is the same divergence of "Evolution Canyon" I strains vs. Dead Sea water strains, and some divergence between "African" and "European" slopes appears. "African" slope and "European" slope populations are significantly different in growth rates under 40 % salinity and have a tendency to be different under 45 % and 50 % volumes of Dead Sea water. We conclude that the *A. niger* group isolated from the Dead Sea water is more resistant, and the "African" slope population has a tendency to be more resistant than the "European" slope to stress associated with low-water activity. We suppose that these patterns are adaptive.

Key words: *Aspergillus niger* group, Dead Sea water, "Evolution Canyon", growth rate, water activity stress

Introduction

Despite numerous studies, the challenging question of how genetic diversity is maintained in nature still remains a focus of modern evolutionary biology. Evolutionarily, it is quite remarkable that stressful conditions may cause higher rates of polymorphism (Nevo 2001), mutation, and recombination (Parsons 1988; Korol *et al.* 1994). Stress-induced genetic variation may serve as an important source of material for natural selection in producing novel adaptations, and differential selection regimes in natural populations may cause complex spatial variation in population structure and level and spectrum of genetic variation (Hoffman & Parsons 1992; Korol *et al.* 1994).

Fungi represent one of the most diverse groups of eukaryotes in the world. Thus, investigating the effect of environmental variability on the diversity of soil microfungi is of great interest, both at the micro- and macroscale levels. Micro- and macrosite ecological contrasts are excellent critical tests for evaluating biodiversity patterns and dynamics and assessing the relative importance of different forces in evolution (Nevo 2001).

*Corresponding author: e-mail: nevo@research.haifa.ac.il

Here we compared and contrasted the growth patterns of the cosmopolitan, filamentous, soil fungi *Aspergillus niger* group from two distinctly different environments: the Mediterranean microsite "Evolution Canyon" ("EC" I) in Mount Carmel, subdivided into two contrasting slopes (xeric "Africa" and mesic "Europe") and the hypersaline Dead Sea (DS), located in the rift valley at the lowest level on the planet (Amiran *et al.* 1985) (Fig. 1).

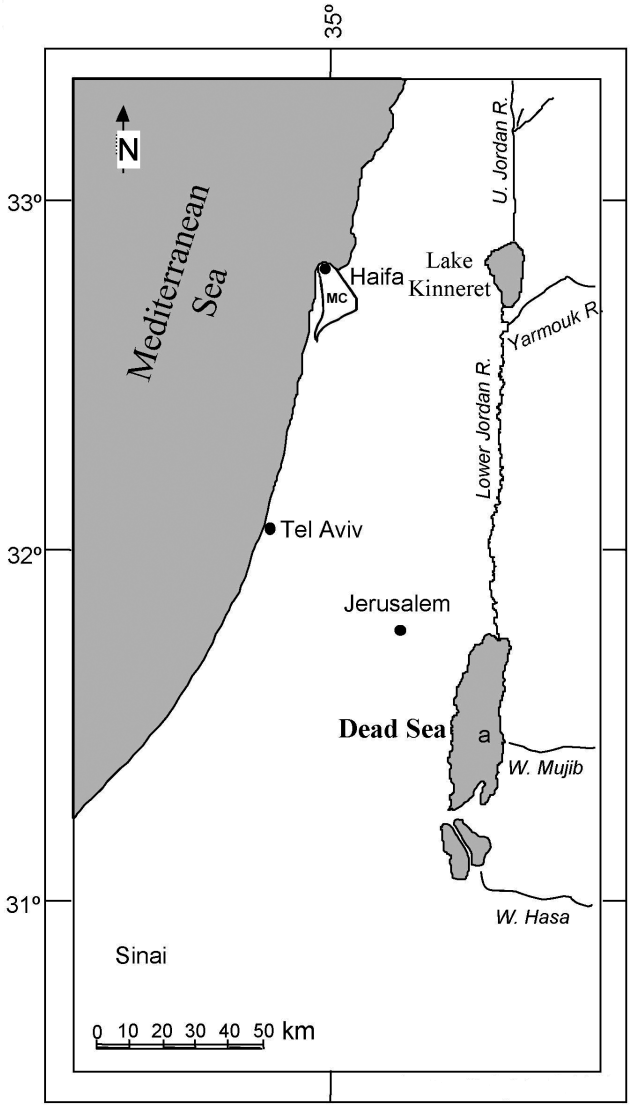

Fig. 1. Map of Israel (Lensky *et al.* 2005): a – Ein Gedi 320; MC – Mount Carmel, the site of "Evolution Canyon"

The opposite slopes of "EC" I at lower Nahal Oren are located at Mount Carmel National Park, Israel. South-facing and north-facing slopes display dramatic biotic contrasts. The south-facing slope ("African", AS) is tropical and xeric, while the north-facing slope ("European", ES) is temperate and mesic. Higher solar radiation on the south-facing slope makes it warmer, drier, and spatiotemporally more heterogeneous than the north-facing slope. Consequently, local biodiversity differentiation across several hundred meters displays global patterns of divergence (Nevo 1995, 1997, 2001).

In "EC" I, problems of biodiversity evolution have been intensively investigated theoretically (Nevo 1995, 1997, 2001, 2007) and factually on different groups across phylogeny: cryptogamic plants, insects, vascular plants, bacteria, and soil microfungi.

The regional contrast in this study was the Dead Sea. Archaea and filamentous fungi have been recorded in the DS (Nevo *et al.* 2003). These microorganisms are adapted to life at high-salt concentrations and to the high-osmotic pressures resulting from the high salinity in their environment.

The DS is potentially an excellent model for studies of evolution under extreme environments and is an important gene pool for future agricultural genetic engineering prospects. The genetic resource of the DS fungi might be a very promising resource to improve salt tolerance in other organisms in relation to various biological systems, including saline agriculture (Jin *et al.* 2005).

As water activity is a critical factor affecting the growth and metabolism of fungi (Scott 1957; Marin *et al.* 1998), numerous investigations have been performed on the effect of water activity and salt concentrations on the germination and growth of different microfungal species. For these investigations, fungi from environments with low-water potential and hypersaline environments (e.g., saline water, saline soil) were tested (e.g., Magan 1997; Kis-Papo *et al.* 2003; Gunder-Cimerman *et al.* 2004). Previous comparative studies of adaptive patterns of soil mycobiota in different Israeli regions showed that environmental conditions strongly influenced the microfungal communities' structure both on regional and local scales (Grishkan *et al.* 2000, 2003a, b, 2004). Following our previous studies, we hypothesize that salt resistance will be different between DS populations of the *Aspergillus niger* group with populations from "EC" I, on a regional scale. Furthermore, we hypothesize that interslope differential salt tolerance will be found on the opposite xeric and mesic slopes of "EC" I displaying interslope differential adaptations. Moreover, we also hypothesize that besides differential adaptations in DS, AS, and ES, incipient sympatric speciation may be ongoing in those dramatically different regional and local habitats (Nevo 2007).

Within the performed study, growing rates of the *A. niger* group from different contrasting localities in Israel under different salt stress were examined and an interpopulation comparison was made. The results revealed presumably adaptive differences on macro- and microscales in the growth rates of the strains from different localities with the change in water activity.

Methods

Study areas

(i) The DS is located in the Syrian-African rift valley between Israel and Jordan. The climate is very arid, with a mean annual rainfall around 60 mm and mean temperatures of the hottest and coldest months from 32-35 °C and 14-16 °C, respectively (Amiran *et al.* 1985). The Dead Sea water (DSW) contains 340 g/l total dissolved salts (Stiller & Nissenbaum 1999). It has very low-water activity (< 0.669 a_w) with a relatively low pH (5.5-6.0) (calculated according to Krumgalz & Millero 1982). The increased, sharply ongoing drying of the DS can be observed nowadays. Divalent cations (Mg^{+2} and Ca^{+2}) now dominate over monovalent cations (Na^+ and K^+). Cl^- makes up 99 % of the anion sum.

(ii) "EC" I (Fig. 2) is located at lower Nahal Oren, Mt. Carmel National Park, Israel (32°43' N, 34°58' E). "EC" I has two opposite, sharply different slopes: the south-facing (tropical, xeric, AS) and north-facing (temperate, mesic, ES) slopes; the distance between slopes is 100 m at the bottom and 400 m at the top. The difference in distribution of solar radiation, which is more intensive on the AS (up to 800 %) than on the ES, caused tremendous physical and biotic contrasts (Pavlicek *et al.* 2003) (Fig. 2). Soils of "EC" I do not contain very soluble salts (such as NaCl and $MgCl_2$), they can be found only in desert conditions (for instance, southern Israel) (Ravikovitch 1992; Rowell 1994). Moreover, the study of chemical composition of soil of "EC" I demonstrated that the sum of Cl^- anions was negligible and no significant difference in its quantity was found between the two slopes. The same study showed that soil of the AS has a pH of a 7.5, ES – 8.0 (Nevo *et al.* 1998). Mean August and January temperatures are 28 °C and 13 °C, respectively, with a difference of 0.7 °C till 3 °C between slopes (more at AS) (Pavlicek *et al.* 2003).

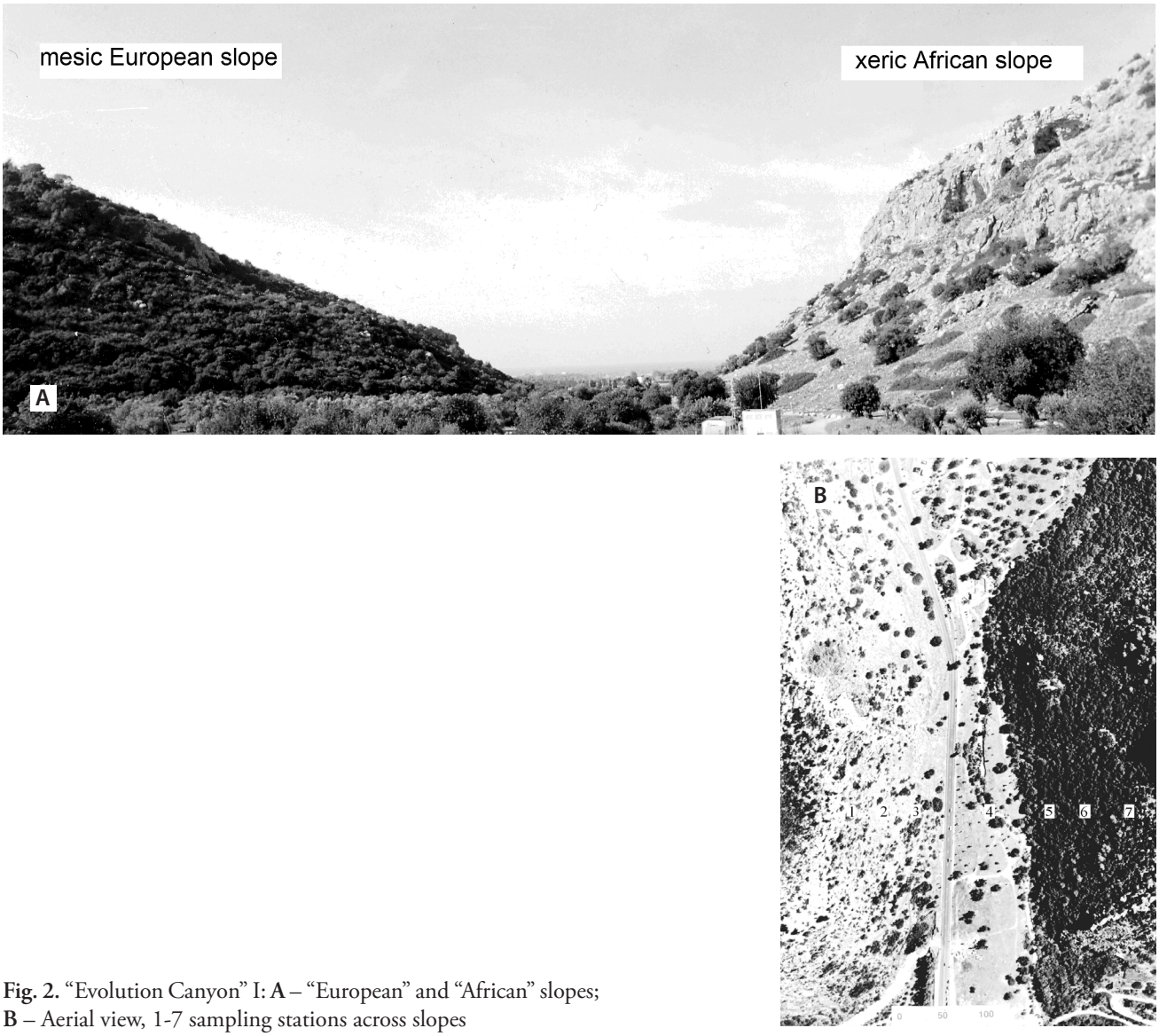

Fig. 2. "Evolution Canyon" I: **A** – "European" and "African" slopes; **B** – Aerial view, 1-7 sampling stations across slopes

Sampling scheme

Samples were collected as follows:

(i) The DSW – from its depth (50 m and 250 m)

Samples for strain isolation were collected in autumn (October) 2005. They were withdrawn from deep water and surface water from the center of the sea, about 8 km northeast of En Gedi ("En Gedi 320") (Fig. 1). Samples were collected by means of Go-Flo sampling bottles and by pumping through a hose (Nevo *et al.* 2003).

Afterwards, water for the experiments was collected the same way four times per year at the same locality.

(ii) "EC" I

Fourteen soil samples from "EC" I were collected from the middle stations of each slope (AS and ES). Samples were collected in shady niches under trees (ES) and in sunny open niches (AS), from the upper soil layer (at 1-5 cm depth). Altogether, 28 samples were collected in spring (April) 2005.

Isolation of strains of Aspergillus niger *group*

Strains of the *A. niger* group were isolated using the soil dilution plate method (Davet & Rouxel 2000). The dilution for the samples was 1:10 by weight. Ten grams of each soil sample collected were suspended in sterile water.

Previous studies in EC I showed that the *A. niger* group was more successfully isolated at 37 °C (Grishkan pers. comm.), such conditions were selective for isolation of the *Aspergillus niger* group. Thus, Petri dishes with sample suspensions were incubated at 37 °C.

For isolation of fungi from the DSW, 2 ml of the DSW (from the depth and surface) were poured into Petri dishes and mixed by rotation with molten agar media (MEA) (30 g/L Malt extract, 3 g/L peptone, 15 g/L agar; pH 6-6.5). The temperature of incubation was the same, 37 °C.

In order to prevent air/lab contamination or cross-contamination of the samples, all inoculated Petri dishes were covered with parafilm and put into plastic bags, individual bags for every sampled locality.

After seven days of incubation, Petri dishes were studied carefully and colonies that were identified as the *Aspergillus niger* group (according to Raper & Fennel 1965) were transferred to the new Petri dishes with MEA for purification and storage. Pure colonies were grown in an incubator at a temperature of 30 °C.

Eighteen strains were isolated for this experiment. Six strains were from the middle station of the AS, № 2 (from sunny, open niches); six from the middle station of the ES, № 6 (from shady niches, under trees and bushes), and six from the DSW, two strains from the surface, distant from the shore, 1 strain from 50 m and 3 strains from 250 m.

Water activity vs. different volumes of Dead Sea water

In the present experiment, the water activity was modified with calculated amounts of DS and distilled water. Water activity of DSW is 0.669 a_w and of distilled water is 1 a_w. Calculations were made according to the following formula:

$$a_w = ((100 - X) * 1 + 0.669\ X) / (100)$$

a_w – water activity
X – used volume of the DSW

Experimental design

It has been previously shown (Trinci 1969; Trinci & Collinge 1973) that the linear growth rate on solid substrate, e.g., agar medium, is a good approximation of biomass increase in liquid culture. In order to check growth rates of different strains of the *A. niger* group under different salt stress, strains were grown at 30 °C on Petri dishes with 10-12 ml of yeast extract glucose agar (GY) (10g/L glucose, 1 g/L yeast extract, 20 g/L agar; pH 6-6.5). Agar disks (4-5 mm in diam) were cut out from 4-day-old cultures on agar plates and inoculated at the center of Petri dishes with GY (35 g/L agar), prepared with different concentrations of DSW (from 0% till 80% volumes of DSW, with increments of 5% volumes of DSW); each strain in two repetitions. Fungi were grown at 30 °C on Petri dishes with GY, prepared in the exact volumes of the Dead Sea water (Table 1).

Table 1. Volumes of Dead Sea water and corresponding water activity used in the experiment

Volumes of Dead Sea water (% v/v)	0	5	10	15	20	25	30	35	40
Water activity,aw	1	0.983	0.967	0.95	0.934	0.917	0.9	0.884	0.868
Volumes of Dead Sea water (% v/v)	45	50	55	60	65	70	75	80	100
Water activity, a_w	0.851	0.835	0.818	0.801	0.785	0.768	0.752	0.735	0.669

In the case of the medium prepared without Dead Sea water, MEA was used. For the media prepared with 10% volumes of DSW, both GY and MEA were used. Measurements of the mycelium growth were recorded in two orthogonal directions every 24 hours until the Petri plates were completely colonized. In treatments with 50% DSW, measurements were stopped on Day 24; colonies didn't reach the end of the Petri dishes, however, the log-phase was already observed.

Data analysis

(i) Mann-Whitney U test

A standard non-parametric Mann-Whitney U test was performed with the use of program "Statistica 6.0".

(ii) Non-parametric Kolmogorov-Smirnov test

The distribution of daily growth rates over all days of growth for all individual colonies of a species was used as the characteristic of species adaptation to salinity levels. This is an integral characteristic that is very sensitive to all, including minor interspecies divergences in growth rates.

For every colony the daily growth rate was calculated as the distance between radii of the colony at day i+1 and day i. There were two measurements per day/colony in two orthogonal directions.

The cumulative distribution of growth rates for a species under each level of salinity was prepared as a series of fractions of rates. All rates of each fraction are less than the fraction-specific threshold, and these thresholds are arranged in increasing order.

Under the same salinity treatment, divergence between species-specific cumulative distributions was checked by the non-parametric Kolmogorov-Smirnov test (fon Mises 1997). The *p*value of the test was computed with the following statistics:

$$\sqrt{N} \cdot \max_i (S(x_i) - F(x_i))$$

that has Kolmogorov distribution.

Here N is the number of thresholds in the series

x_i is *i-th* threshold of growth rates

$S(x_i)$ – the fraction of rates of the first distribution that are less than threshold x_i

$F(x_i)$ – the fraction of rates of the second distribution that are less than threshold x_i

Results

The minimum water activity growth limit was 0.785 (65 % of volumes of DSW). After two months of incubation at 30 °C, none of the strains showed any growth in the range of 0.785 – 0.735 a_w (65 % - 80 % of volumes of DSW). In the range of 0.818 – 0.801 a_w (55 % - 60 % of volumes of DSW) colonies grew very slowly; therefore, no data on measurements could be obtained.

Two methods were applied to detect any differences at the micro- and macroscale. First, we used statistical standard non-parametric Mann-Whitney U test. Significant differences between "EC" I and DSW growth rates we observed at 1, 0.983, 0.967 and 0.868 a_w (0 %, 5 % and 40 % volumes of DSW, correspondingly, $p<0.005$). No significance between growth rates of populations (neither at macro- nor at microscale levels) was observed under any other treatment when we used Mann-Whitney U test (Fig. 3).

However, the trend for differences in the growth rates between populations was very clear: all mean, median, and maximum values of the strains isolated from DSW were obviously different from those that were calculated for "EC" I (both slopes) isolates. Also, we observed some trend for differences within two slopes of "EC" I under extreme low-water activities. Therefore, we decided to apply non-parametric Kolmogorov-Smirnov test (fon Mises 1997), which is considered to be a more precise and accurate statistical analysis.

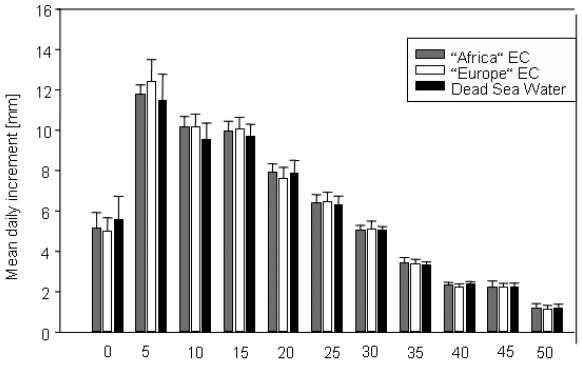

Fig. 3. Average growth increment of DSW and "EC" I ("AS" and "ES") populations across all salinities

Indeed, when using non-parametric Kolmogorov-Smirnov test we got a more detailed picture that confirmed previous results. At the control, 1 a_w, fungi from DSW demonstrated significantly higher activity in growth compared with all "EC" I strains. Strains of the *A. niger* group at the control were grown on the MEA. As MEA prepared with larger volumes of DSW did not solidify, we used GY instead. In order to be sure that the difference in the strains' behavior was not influenced by the type of media used in the experiment, the experiment with 10 % volumes of the DSW (0.967 a_w) was repeated with MEA for all 18 strains. Distribution in the growth rate was the same as we observed on GY medium prepared with 10 % volumes of DSW (0.967 a_w).

Optimal growth for all strains was observed at 0.983 a_w (5 % of volume of DSW). However, significant interpopulation growth differences were observed between populations from different environments (AS, ES, and DSW). Fungi isolated from "EC" I grew distinctly faster than those isolated from DSW. The Kolmogorov-Smirnov test revealed a statistically significant *p*value (1E-08) for differences in the growth rates (Table 2). The same differences and growth rate distributions were observed at 0.967 and 0.95 a_w – 10 % and 15 % of DSW volume, correspondingly. On media with these salinities, fungi from "EC" I grew significantly faster than strains from the DS.

Table 2. Kolmogorov-Smirnov test *p*-values for pairwise differences between cumulative distributions of growth rates

Comparison of populations	Volumes of Dead Sea water (% v/v)										
	0	5	10	15	20	25	30	35	40	45	50
	p-values										
ES – AS	0.9	0.81	0.76	0.45	0.63	0.56	0.45	0.24	0.001	0.1	0.06
ES – DSW	1E-09	1E-08	0.002	2E-04	0.25	0.3	0.63	0.45	1E-06	1E-04	1E-06
AS - DSW	1E-08	1E-08	0.003	3E-04	0.25	0.3	0.56	0.63	0.001	1E-04	1E-06

An increase in volumes of DSW resulted in a change in this pattern. At water activities of 0.934 – 0.884 a_w (20 % - 35 % volumes of DSW), no significant differences between the populations' growth rates were observed. However, when a_w reached 0.868 (40 % volume of DSW), salt-tolerant strains from the Dead Sea started to show higher growth rates again. The same pattern was maintained at lower water activity - 0.851 and 0.835 a_w (45 % and 50 % volumes of DSW).

Summarizing all of the abovementioned results, it can be stated that fungi from the EC grew significantly faster at high-water activity (0.983 - 0.95 a_w; 5 % - 15 % volumes of DSW), while fungi from the DS grew faster at the extremes both at the highest, 1 a_w (0 % volumes of DSW) and the lowest activity allowing growth, 0.867 - 0.835 a_w (40 % - 50 % volumes of DSW). In medium with moderate water activity ranges (0.934 - 0.884 a_w; 20 % - 35 % volumes of DSW), no significant differences in growth rates were observed between the DS and "EC" I populations (Table 3).

Table 3. Differences in growth between populations from the Dead Sea and "Evolution Canyon" I ("African" (AS) and "European" (ES)) under differential stressful influence of different a_w

Water activity	DSW volumes (% v/v)	Growth rates
0.99	0	DS>EC
0.974 – 0.942	5 – 15	EC>DS
0.926 – 0.878	20 – 35	EC=DS
0.862 – 0.829	40 – 50	DS>EC
0.813 – 0.796	55 – 60	Very slow – all populations
0.779	65	No growth – all populations

The growth rate differences were detected not only on the macroscale level ("EC" I vs. DSW), but also at the microscale level ("AS" vs. "ES" of "EC" I). While high and moderate water activities (1 - 0.884 a_w; 0 % - 35 % volumes of DSW) didn't result in any significant interslope differences in the growth rate, "AS" and "ES" populations were significantly (by KS test) different in growth rates at 40 % of salinity (0.867 a_w) and had a tendency to be different at 45 % and 50 % volumes of the DSW (0.851 and 0.835 a_w). Population isolated from "AS" grew significantly (KS test) better under very low-water activity (0.867 a_w; 40 % volumes of DSW) and obviously better on the media with 0.851 and 0.835 a_w (45 % and 50 % volumes of the DSW), compared to the population isolated from "ES" of "EC" I (Table 2). In other words, the population from xeric "AS" tended to resemble population isolated from extremely xeric and salty DS.

Discussion

The present experiment is one of a very few studies, in which ecological comparisons have been made between the strains of fungi from such contrasting environments as the hypersaline Dead Sea and the Mediterranean "Evolution Canyon" I. Moreover, comparison has been provided not only at the macroscale but also at the microscale level ("AS" vs. "ES").

The present study provided evidence indicating that the DS *A. niger* group is different from that of "EC" I. Moreover, some interslope divergence at "EC" I may suggest, as in other taxa from bacteria to mammals (Nevo 1997, 2001, 2007), that the cosmopolitan *A. niger* group might undergo incipient sympatric speciation at "EC" I, and probably more advanced speciation at DS.

The study sites

DSW

Mycological studies of the water samples from the DS revealed soil mycobiota comprising 77 species from 26 genera (Nevo *et al.* 2003). The general composition of filamentous fungi demonstrated a great number of melanin-containing microfungi (36 %), a marked number of *Aspergillus* (27 %), and to a lesser degree – *Penicillium* representatives (19 %) and ascomycete teleomorphic fungi (17 %). Such a composition can be considered a characteristic for mycobiota of a highly stressful environment (Grishkan *et al.* 2003a; Nevo *et al.* 2003).

DSW indigenous and foreign fungal species

Some studies were provided previously on spores and mycelia of DS isolates to their hypersaline environment. A comparison was made of their behavior with that of isolates of the same species or of closely related ones, from the DS terrestrial shore and from a control Mediterranean environment. A comparison showed prolonged viability of the isolates obtained from the DSW. Spores survived after 3 months in undiluted and diluted DSW; mycelium retained their viability in DSW diluted to 80 % during the 12 weeks of the experiment, and 8 weeks in undiluted DSW. Spores and mycelium of isolates obtained from the terrestrial shore of the DS generally proved less tolerant to suspension in undiluted DSW. They demonstrated only 10-20 % spore survival after 12 weeks; none of the isolates survived more than 6 weeks in undiluted DSW. Spores and mycelium of the species isolated from the control sites had lost their viability in undiluted DSW before the end of the experiment (spores survived less than 8 weeks; mycelium retained viability less than 6 weeks in undiluted DSW) (Kis-Papo *et al.* 2003).

The durability of both spores and mycelia of the DS isolates is important as an adaptation of fungi to life in the hypersaline waters of the DS, where development of mycelium may be possible only during the rare episodes in which the salinity of the upper water layers becomes reduced as a result of massive inflow of fresh water.

The Dead Sea has a constant inflow of fungal hyphae and spores carried by wind, rain, and runoff. The Jordan River and the nearby springs may cause further intrusion; migration of fungi between the shore and the water column is also probable. Undiluted DSW does not support growth of any known fungal species. However, areas near freshwater springs or the Jordan River estuary may be diluted havens. It is reasonable to assume that DSW isolates may complete their life cycle in the spatially-temporally-formed diluted area (Nevo *et al.* 2003).

Concluding the above, the potential for the introduction of foreign fungal species into the DS is very low.

"EC" I

Slopes of "EC" I are separated by 100 m at bottom and 400 m at top. Mycological studies in "EC" I revealed soil mycobiota comprising 204 species (Ellanskaya *et al.* 1997; Grishkan *et al.* 2000). The results demonstrated a strong interslope impact of edaphic and climatic conditions on species richness, composition, and abundance.

Incipient speciation of some species isolated from "EC" I was shown in different studies. For instance, the genomic and physiological characteristics of the *Bacillus simplex* metapopulation in "EC" I are clearly nonrandom and display strong interslope and intraslope divergence shaped by natural selection overriding migration patterns and leading to adaptive incipient sympatric speciation (Sikorski & Nevo 2005). Moreover, preliminary interstrain cross-fertility tests were performed on cultures of the teleomorphic fungus *Sordaria fimicola* from "EC" I as well as from widely separated areas in Israel, America, and Canada. The tests demonstrated excellent cross-fertility within strains from the same "EC" slope, an occasional lack of cross fertility within strains from different slopes, and no cross-fertility within strains from different geographical areas (Lamb *et al.* 2000).

The experimental model

Examining and comparing species isolated from the Dead Sea to the same species from other environments can answer the question as to what extent have the species isolated from the Dead Sea adapted to its hypersaline brine habitat (Kis-Papo *et al.* 2003) and even may have started to speciate into a salt-resistant species.

Furthermore, this is the first study in which a complicated mixture with large amounts of different chemical elements, such as with DSW, was used for adapting a_w in a growth rate study. Previously, in all similar experiments on growth rates, the water activity of the medium was modified with calculated amounts of the non-ionic solute glycerol and the ionic solute NaCl. The use of glycerol to modify media water availability produced a higher growth rate when compared to NaCl, probably because glycerol can be utilized as a carbon source and can act directly as a compatible solute. In contrast, high concentrations of NaCl can be toxic, and this may explain the differential growth patterns observed (Parra *et al.* 2004). These studies clearly showed that the wild-type strains grew optimally at 0.98 - 0.93 a_w on glycerol-modified media, but optimally at 0.99 a_w when NaCl was used (Parra *et al.* 2004; Su-lin *et al.* 2006). All the strains grew in CYA (Czapek Yeast Extract) medium from 0.86 to 0.99 a_w, and three of them also grew in YES (Yeast Extract Sucrose) medium adjusted to 0.82 a_w (with glycerol) from 20 days of incubation (Esteban *et al.* 2006). Slow growth of *A. niger* isolates was observed at 0.76 a_w on malt extract agar (MEA) (Vujanovic *et al.* 2001), whereas in another study the minimum water activity limit for growth was 0.82 using the same culture medium (Parra *et*

al. 2004). We conducted an experiment modifying medium with natural environment (DSW) in order to get results on the adaptation of fungi to their habitats and to clarify growth rate differences between populations from different habitats. The water of the Dead Sea contains 340 g/l total dissolved salts (Stiller & Nissenbaum 1999); divalent cations (Mg^{+2} and Ca^{+2}) dominate over monovalent cations (Na^{+} and K^{+}); Cl^{-} makes up 99 % of the anion sum. Therefore, it was impossible to predict fungal growth rates on the medium prepared with different volumes of DSW.

Previous experimental studies on the combined influence of water activity and temperature on growth and spore production of *Aspergillus niger* strains isolated from food demonstrated that the strains studied grew optimally at 0.970 - 0.980 a_w at temperatures between 30 °C and 37 °C (Belli *et al.* 2004; Parra *et al.* 2004). Our data confirm this statement. The optimum growth for all strains in our research was not at 1 a_w but rather a lower water activity, 0.983 a_w (5 % volumes of DSW).

Using the non-parametric Kolmogorov-Smirnov test, we found that strains originating from "EC" grew significantly faster at high-water activity (the a_w in the range of 0.983 - 0.934), while those originating from the Dead Sea grew faster at extremes - low activity allowing growth, 0.867 - 0.835 a_w and high-water activity, 1 a_w.

These intriguing and unexpected results can be explained by the following hypothesis. As the *Aspergillus niger* group is considered to be xerotolerant, 1 a_w (0 % volumes of DSW) is slightly stressful for these fungi; *A. niger* group from DSW is salt-resistant, and it is adapted to a much lower a_w; such high a_w is much more stressful for this population than for the population from "EC" I. As a result, stress-responsive pathways are activated, and the DSW population grows faster than the one from "EC" I. While low and "moderate" concentrations of the DSW (5 % - 35 % volumes of DSW; 0.983 - 0.884 a_w) are not that stressful for the salt-resistant *A. niger* group from DSW. Therefore, none of the stress-responsive pathways are activated and populations grow slower or equally, compared to populations from "EC" I. Nevertheless, the strains isolated from "EC" I 5 % - 15 % volumes of DSW (0.983 - 0.95 a_w) are as stressed as 0 % (1 a_w) for the DSW strains. Consequently, all the mechanisms to resist such stress are activated, and populations from "EC" I obviously grow faster. Moderate water activities of 0.934 - 0.884 a_w (20 % - 35 % volumes of DSW) are already much more stressful for the *A. niger* group from "EC" I, but still not as stressful for DSW's population. As a result, all populations grow with the same speed. Likewise, at 0.867 - 0.835 a_w (40 % - 50 % volumes of DSW), the influence of stress is too high for strains from "EC" I and is already enough to generate responses for strains isolated from the Dead Sea water. Therefore, DSW strains grow significantly faster.

The "Evolution Canyon" I model

Microscale comparison also revealed interpopulation differences in the growth rates. Significant divergence between xeric "AS" and mesic "ES" of the "EC" I was found. "AS" and "ES" populations are obviously different in growth rates under 40 % of salinity (0.867 a_w) and have a tendency to be different under 45 % and 50 % volumes of Dead Sea water (0.851 - 0.835 a_w). Populations from xeric "AS" have higher growth rates than populations from the mesic "ES", approaching DS growth rates. The interslope differences can be explained as follows. "AS" is tropical and xeric; the population isolated from it is more adapted to drought stress. By contrast, "ES" is mesic and temperate; its strains are less adapted to drought stress. As a result, the *A. niger* group from the "AS", at extremely low-water activities, has higher growth rates than the one isolated from "ES" and is more similar to DS strains than to its counterpart 200 m apart.

In summary, we identified interpopulation differential resistance in accordance with the ecological stress of their original habitat. Due to the very high, constant salt stress of the habitat, to which strains from the DS are subjected, strains are more resistant to the stress associated with low-water activity. Results clearly show that within the hypersaline DS, evolutionary adaption and speciation processes have occurred during the increasing salinization of the DS over its 70,000 years of existence and primarily during the last 15,000 years. Moreover, in "EC" I we may witness incipient speciation at a microscale of the *Aspergillus niger* group on the opposite slopes as in other taxa across phylogeny (Nevo 2001).

Prospects

Obviously, additional investigations must be performed to confirm or disprove the results presented here. Therefore, molecular based experiments are currently planned (i.e., wide genome gene expression analysis surveying thousands of genes across the genome). This will provide insights into the extent of genomic divergence of strains of the *Aspergillus niger* group from the DSW and "EC" I (including both slopes).

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