

Extremotolerance in fungi: evolution on the edge

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Abstract

Our planet offers many opportunities for life on the edge: high and low temperatures, high salt concentrations, acidic and basic conditions and toxic environments, to name but a few extremes. Recent studies have revealed the diversity of fungi that can occur in stressful environments that are hostile to most eukaryotes. We review these studies here, with the additional purpose of proposing some mechanisms that would allow for the evolutionary adaptation of eukaryotic microbial life under extreme conditions. We focus, in particular, on life in ice and life at high salt concentrations, as there is a surprising similarity between the fungal populations in these two kinds of environments, both of which are characterized by low water activity. We propose steps of evolution of generalist species towards the development of specialists in extreme habitats. We argue that traits present in some fungal groups, such as asexuality, synthesis of melanin-like pigments and a flexible morphology, are preadaptations that facilitate persistence and eventual adaptation to conditions on the ecological edge, as well as biotope switches. These processes are important for understanding the evolution of extremophiles; moreover, they have implications for the emergence of novel fungal pathogens.

Introduction

Environments with extreme physicochemical parameters were thought of as being hostile until microbiologists discovered that they are actually inhabited by a wide diversity of microorganisms. Organisms that survive and thrive under conditions that are detrimental to the majority of other species have become a focus of increasing scientific attention over the last few years, with some groundbreaking discoveries of stress-tolerating mechanisms. Extremophiles are promising models to further our understanding of the functional evolution of stress adaptation. Their biology widens our views on the diversity of terrestrial life and it has come as a surprise that not only prokaryotes but also eukaryotes have a great capacity to adapt to extreme conditions. Particularly successful examples can be found in the fungal kingdom. Thus, specialized fungi have been discovered in extreme cold, dry, salty, acidic and deep-sea habitats. How did this capacity evolve?

Comparative results of evolutionary studies have helped us to better understand the adaptability of extremophiles. Shared patterns in morphology, phylogeny and population characteristics have been seen in studies of fungi from extremely cold or salty environments. In particular, the

importance of small populations at the geographical or ecological periphery is now better appreciated, as these can shelter unique adaptations (Alleaume-Benharira *et al.*, 2006; Johannesson & Andre, 2006). In this review, we summarize the current knowledge of the evolution of fungi that inhabit extreme habitats. We also develop a hypothesis that can explain this evolution of extremotolerance in fungi. In doing so, we focus on glacial and hypersaline environments, although we also include examples of emerging pathogenic fungi in stress-tolerant phylogenetic lineages.

Glacial habitats

Ice in nature has long been considered as only enclosing microorganisms that have been randomly deposited on its surface (Ma *et al.*, 2000). However, it is now known that different types of ice provide environments that can support active microbial growth and reproduction, such as for snow, glacial ice and sea ice (Christner *et al.*, 2000; Price & Sowers, 2004; Rohde & Price, 2007; Price, 2009). In particular, several fungal species have been isolated in considerable numbers from subglacial ice of polythermal glaciers (Gunde-Cimerman *et al.*, 2003; Butinar *et al.*, unpublished data).

Among these, there are cosmopolitan species belonging to common mould genera that do not, at first glance, look much different from isolates from elsewhere. However, a study that included molecular analyses of *Penicillium crustosum* populations isolated from glaciers on the island of Spitsbergen (Svalbard, Norway) demonstrated that the majority of these Arctic isolates cluster into two main groups that are distinct from strains isolated in other parts of the World (Sonjak *et al.*, 2009). One of these groups cannot use creatine as the sole carbon source and produces a secondary metabolite, andrastin A, two properties never found previously in this species (Sonjak *et al.*, 2007a). Another *Penicillium* species is closely related to the primarily temperate *Penicillium piscarium* and *Penicillium simplicissimum*, and it differs in the production of secondary metabolites and in the morphology of its conidia and penicilli; it has been named *Penicillium svalbardense* (Sonjak *et al.*, 2007b). Other potentially new *Penicillium* species that have also been found in the same environment still await description (unpublished data).

Aureobasidium pullulans is an osmotolerant black yeast (Kogej *et al.*, 2005) that has biotechnological importance due to its production of pullulan (Leathers, 2003; Singh *et al.*, 2008). Its hyphae and yeast cells can adhere to moist surfaces and thrive in a wide range of habitats (Webb *et al.*, 1999), including the phyllosphere (Andrews *et al.*, 2002), polluted water (Vadkertiova & Slavikova, 1995) and solar salterns (Gunde-Cimerman *et al.*, 2000). Recently, *A. pullulans* has also been isolated from Arctic glaciers and from sea water in Kongsfjorden, on Spitsbergen, Svalbard, Norway (Zalar *et al.*, 2008b). *Aureobasidium pullulans* has a relatively broad amplitude of ecological tolerance, and it can survive hypersaline (Gunde-Cimerman *et al.*, 2000), acidic and basic (Ranta, 1990; Shiomi *et al.*, 2004) conditions, and low temperatures and nutrient concentrations (Onofri, 1999). Adaptations of *A. pullulans* to extreme environments have been described at the molecular level (Kogej *et al.*, 2005, 2006; Turk *et al.*, 2007; Gostinčar *et al.*, 2008). Environmental stress is mitigated by rapid dimorphic switching from small colourless yeast cells to thick-walled, heavily melanized, meristematic forms (Bermejo *et al.*, 1981). Dry cells are easily distributed in the air (Colakoglu, 2003; Lugauskas *et al.*, 2003). Wet cells have a water-soluble coat of extracellular polysaccharides (Kim *et al.*, 2000). A multilocus phylogenetic comparison of *A. pullulans*-like isolates from Arctic glaciers and their immediate surroundings that included a worldwide selection of strains has revealed that the globally distributed strains of *A. pullulans* belong mainly to two multilocus genotypes, for which two older varieties (var. *pullulans* and var. *melanogenum*) have been restored (Zalar *et al.*, 2008b). The *pullulans* variety was found mostly in mildly osmotic environments, while the *melanogenum* variety was isolated mainly from oligotrophic watery habitats. Both of these varieties have also been found in

Arctic habitats. Sequence data of five molecular markers revealed an additional genotype that was only isolated from the subglacial ice of the Kongsvegen glacier and its immediate surroundings. This genotype was described as *A. pullulans* var. *subglaciale* (Zalar *et al.*, 2008b). The existence of these three distinct varieties is supported by considerable genealogical congruence among the gene loci. These patterns suggest a strictly asexual life style and reproductive isolation.

Species of the genus *Thelebolus* tend to be psychrophilic (Wicklow & Malloch, 1971). *Thelebolus microsporus* occurs globally in boreal climate zones, while in the extreme climate of the Antarctic, it has evolved into two endemic genotypes; these have a strongly reduced morphology and cannot undergo sexual interactions. These two genotypes were described as novel species: *Thelebolus ellipsoideus* and *Thelebolus globosus* (de Hoog *et al.*, 2005a). This segregation was explained by a loss of a bird vector and the subsequent life under perennial ice, as follows: *Thelebolus* species are consistently dung- or guano-associated, and thus they have an ecological advantage as they remain close to mammal or bird vectors for their dispersal. For this, they form sticky ascospores that are forcibly discharged to reach the body of the host. Conidia are not needed; they are absent from all of these vectored species. The Antarctic species mentioned above, however, are believed to have become trapped under near-perennial ice in an environment without birds or mammals. Hence, their asci degenerated and can no longer undergo forcible spore discharge. Instead, conidia were produced for water-borne dispersal. These dramatic mechanistic and morphologic changes developed over a very short time frame, as the multilocus sequence data studied hardly show variability (de Hoog *et al.*, 2005a).

Saline habitats

Fungal survival and proliferation have been regarded as improbable in hypersaline environments such as water in evaporation ponds of solar salterns. However, studies of fungal populations in natural hypersaline environments on several continents have revealed the abundant and consistent occurrence of several specialized fungal species (Gunde-Cimerman *et al.*, 2000). These species are characterized by extensive and complex molecular adaptations to low water activities and high concentrations of toxic ions (for reviews, see e.g. Gunde-Cimerman *et al.*, 2005a, b; Plemenitaš *et al.*, 2008).

Strains identified as *Cladosporium sphaerospermum* have been consistently isolated from hypersaline environments around the globe (Zalar *et al.*, 2007). *Cladosporium sphaerospermum* is one of the most common air-borne, cosmopolitan *Cladosporium* species, and it has been frequently isolated from indoor and outdoor air (Park *et al.*, 2004) and dwellings (Aihara *et al.*, 2001), and occasionally from humans (Badillet *et al.*, 1982) and plants (Pereira *et al.*,

2002). Although this species has also been reported from osmotically nonstressed niches, strains that were morphologically identified as *C. sphaerospermum* can grow at a very low water activity (contrary to other cladosporia; Hocking et al., 1994). Molecular analysis has shown that *C. sphaerospermum*-like isolates were actually a complex of species. *Cladosporium sphaerospermum* was thus redefined, and seven new species from hypersaline environments have been described. Some of these have, to date, been found exclusively in hypersaline waters of solar salterns (Zalar et al., 2007). Similarly, several new species taken exclusively from hypersaline environments have been described for the genera *Eurotium* (Butinar et al., 2005b), *Trimmatostroma* (Zalar et al., 1999a), *Emericella* (Zalar et al., 2008a) and *Phaeothea* (Zalar et al., 1999b).

The black yeast-like fungus *Hortaea werneckii* is one of the most halotolerant fungi, with a broad growth optimum from 1.0 to 3.0 M NaCl (Gunde-Cimerman et al., 2000), and it can grow in nearly saturated salt solutions, as well as without sodium chloride. Hypersaline waters appear to be its primary ecological niche in nature, such as those found in salterns (Gunde-Cimerman et al., 2000; Butinar et al., 2005a). Despite the ability of *H. werneckii* to grow without salt, it has been isolated only occasionally in NaCl concentrations < 1.0 M, while at 3.0–4.5 M NaCl, this species can represent as much as 85–90% of all of the fungal isolates from salterns. *Hortaea werneckii* is well adapted to environments with low water activities through several of its traits: plasma membrane composition, enzymes involved in fatty acid modifications (Turk et al., 2004, 2007; Gostinčar et al., 2009), osmolyte composition and accumulation of ions (Petrovič et al., 2002; Kogej et al., 2005, 2006), melanization of the cell wall (Kogej et al., 2004), differences in the high osmolarity glycerol signalling pathway (Turk & Plemenitaš, 2002) and differential gene expression (Petrovič et al., 2002; Vaupotič & Plemenitaš, 2007b). The only other species known to be a part of this genus is the acid-tolerant *Hortaea acidophila* (Hölker et al., 2004).

All fungi described to date can grow well in media without salt, with one known exception. *Wallemia ichthyophaga* is the most halophilic eukaryote known and it cannot grow in media with NaCl concentrations < 1.5 M. As only two other species belong to the same genus, *Wallemia sebi* and *Wallemia muriae*, this genus is extremely distinct from all fungi known to date, and it forms an isolated lineage at the base of the *Basidiomycota* tree (Matheny et al., 2006). These three species have now been grouped into their own class: *Wallemiomycetes*. *Wallemia ichthyophaga* has been isolated only four times, twice from hypersaline water and twice from salted meat. This species is phylogenetically quite distant from *W. sebi* and *W. muriae* (Zalar et al., 2005; Matheny et al., 2006), as these others do not show any strong preference for NaCl; instead, they prefer high-sugar environments (Zalar et al., 2005).

Ecological species types

According to their abilities to inhabit extreme environments, fungal species can be classified into three ecological groups (Fig. 1):

Mesophiles do not tolerate extreme conditions (Fig. 1, a1). Instead, they proliferate in environments with moderate physicochemical parameters (Fig. 1, a2). Mesophilic species are not always homogenous and they can include strains that tolerate moderate stress (Kvitek et al., 2008), although in nature they predominantly inhabit nonstressed environments.

Generalists tolerate a variety of stressful environments, but not the most extreme conditions. In laboratory cultures, generalists usually grow best under moderate conditions (Fig. 1, b1), although in nature they are often more successful in somewhat stressful environments, due to limited competition with mesophiles (Fig. 1, b2). An example is the above-mentioned *A. pullulans*, although its varieties *pullulans* and *melanogenum* show that even generalist species sometimes adapt by structuring their populations into groups that are better adapted to certain types of environments (Zalar et al., 2008b).

Specialists include species that are moderately to extremely tolerant or even extremophilic (Fig. 1c). Examples here are *H. werneckii*, which grows both under extreme salt conditions and without salt, and *W. ichthyophaga*, which cannot grow without salt, even under laboratory conditions. We can therefore distinguish *adaptive extremophiles* with a broad ecological amplitude (e.g. *H. werneckii*) and *obligate extremophiles* with a narrow ecological amplitude (e.g. *W. ichthyophaga*). Although it is easier to envisage that fungi adapt to a narrow range of ecological conditions, similar to prokaryotic halophiles (Oren, 1999), the narrow-amplitude strategy is much rarer in halophilic fungi. However, despite these differences, both *H. werneckii* and *W. ichthyophaga* are found in hypersaline environments and characterized by growth optima at extreme salinity. The ability of *H. werneckii* to grow without salt is hardly used under natural conditions. A similar pattern of adaptation to a broad amplitude of an ecological factor occurs with respect to temperatures. As with salinity, the growth optimum can be different from that realized in natural habitats. For instance, rock-inhabiting meristematic black fungi from the Antarctic show optimal growth at temperatures significantly higher than those to which they are usually exposed to in their natural environment (Selbmann et al., 2005).

Evolutionary specialization to extreme habitats

We propose the following steps as the evolutionary progression to extremotolerance and extremophily (schematically represented in Fig. 2):

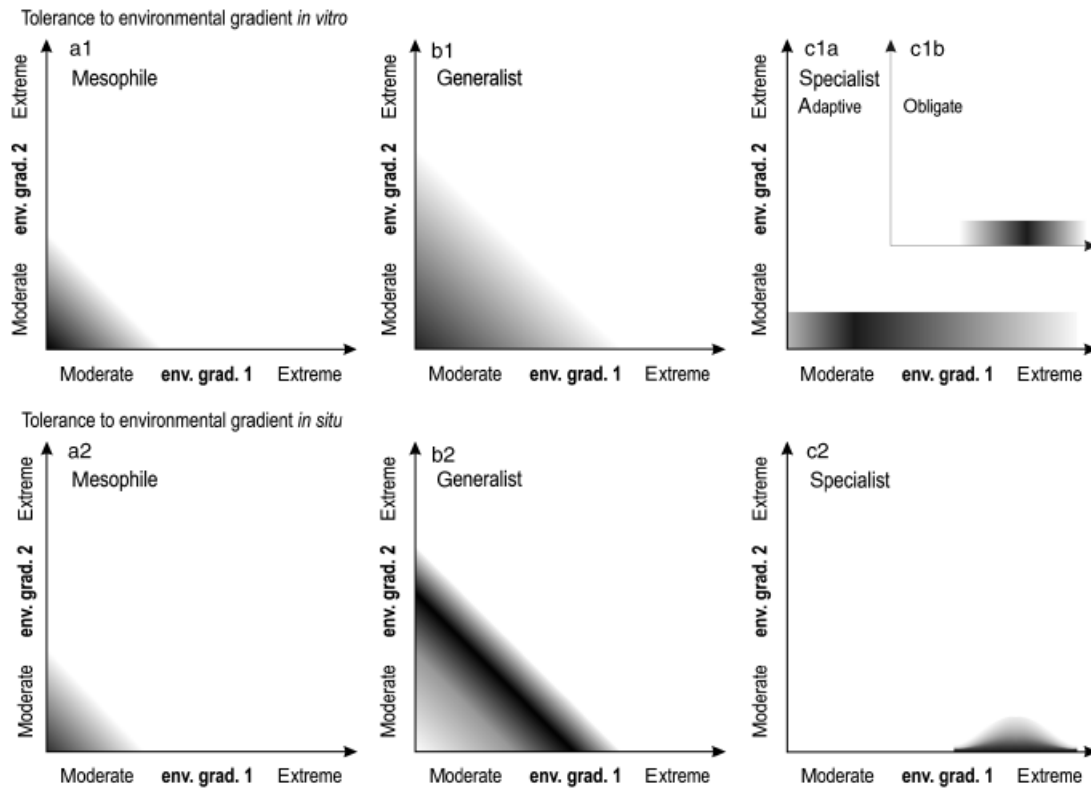


Fig. 1. Schematic representation of three different types of response to an environmental gradient of a specific physicochemical parameter (e.g. water activity). Darker shading represents a greater ability of the organism to thrive at a specific value of the environmental gradient. (a1, b1, c1a, b) Responses to the gradient under laboratory conditions (pure culture). (a2, b2, c2) Responses to the gradient for the range in which the organism is most successful in nature. For simplicity, only two environmental gradients are presented. The proposed classification is as follows: (a1, a2) *Mesophilic* organisms proliferate in environments with moderate physicochemical parameters. (b1) *Generalists* tolerate a variety of stressful environments and have a growth optimum under moderate conditions. (b2) In nature they are often predominantly found in moderately stressful conditions, due to limited competition with mesophiles and their inability to compete with the most extreme conditions. (c1) *Specialists* are extremotolerant or even extremophilic species with their growth optimum shifted towards extreme values. They are either unable to compete with species in more moderate environments (adaptive specialists, c1a) or simply unable to survive moderate conditions (obligate specialists, c1b) and predominantly inhabit extreme habitats (c2).

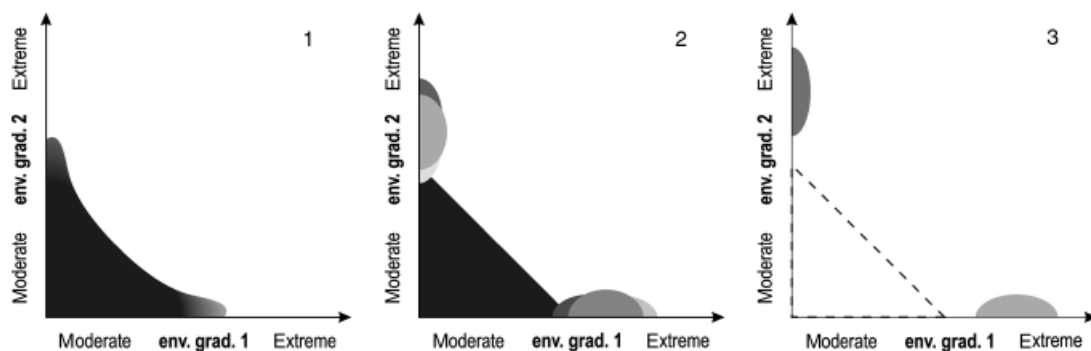


Fig. 2. Schematic representation of the proposed stages in the evolution of extremophilic species: 1, *Persistence*: Generalistic species serve as a genetic reservoir of potential candidates for the evolution of extremophiles. Through their tolerance to stress, the distributional limits of generalist fungal species are usually in extreme environments. Individual organisms react within the limits of their phenotypic plasticity. 2, *Adaptive radiation*: When the conditions at the ecological periphery are stable, genetic fixation of the traits that are beneficial for survival in a certain environment can follow, possibly accelerated by fragmentation of the peripheral habitats (and the consequent bottleneck effects and genetic drift), and even through the accelerated mutation rates. 3, *Specialization*: Further specialization by purification selection can shift growth optima from the ancestral ecological amplitude towards the extremes of the ancestral ranges. In the evolutionary course, the ancestral species can become extinct or diverge too much to be still recognized as being closely related to the specialist species.

Persistence

Generalist species appear to serve as a genetic reservoir of potential candidates for the evolution of extremophiles. *In vitro* studies have shown that generalists are much more likely to diversify than specialists (Buckling *et al.*, 2003). This is possibly aided by the so-called 'robust genotypes', as defined by Agosta & Klemens (2008), which allow persistence across varied environments without obligate adaptation to local conditions.

Apart from the role in persistence of robust genotypes, dispersal efficiency is probably an important determinant for the further separation of individual populations in different habitats. Dispersal efficiency can be species specific. A study of *Saccharomyces paradoxus* showed increasing genetic divergence with geographical distance at different spatial scales (Koufopanou *et al.*, 2006), whereas this was not seen for *Saccharomyces cerevisiae* (Katz Ezov *et al.*, 2006). Generally, we still have limited information on the biogeographical patterns of genetic variation in extremophilic fungi.

Through their tolerance to stress, the distributional limits of generalist fungal species are usually in extreme environments. Fragmentation of permissive habitats at the ecological edge can create small bottleneck populations (Bridle & Vines, 2007) that are exposed to severe selection pressures due to extreme physicochemical parameters. In addition, associated organisms can contribute to selective constraints in fungal habitats (e.g. bacteria; de Boer *et al.*, 2008). Without sexuality and/or gene flow, progressing genetic drift will rapidly fix alleles in small populations that have managed to adapt to extreme habitats. Indeed, many extremophilic fungal species have a mainly mitotic life style. An example is seen in the asexual *A. pullulans*, although even in species with sexual cycles, small isolated populations can diversify independently (e.g. *Eurotium* and *Emericella* species; Butinar *et al.*, 2005b; Zalar *et al.*, 2008a). Phenotypic plasticity is probably of key importance in adaptation to new environments, as argued by West-Eberhard (2005). This corresponds to the concept of 'ecological fitting', as was recently put forward by Agosta & Klemens (2008). Persistent ecotypes are subject to selection for genetic adaptations (West-Eberhard, 2005), and consequently, evolutionary radiation can take place (Agosta & Klemens, 2008).

Dothidealean black yeast-like fungi are a good example of a group that has large phenotypic plasticity: they have thick cell walls that include melanin, which protects them from UV and hyperosmolar stress (Kogej *et al.*, 2007). They are often capable of slow meristematic growth, which possibly enhances their ability to survive in the presence of various stress factors (reviewed in Selbmann *et al.*, 2005). Some of these species produce extracellular polysaccharides that also confer stress tolerance (Selbmann *et al.*, 2005). Species from this group that is characterized by tolerance to extremely

cold and salty environments also tolerate one or more of the following: extreme temperatures (Sterflinger, 1998), desiccation (Gorbushina *et al.*, 2008), low pH (Hölker *et al.*, 2004) and radiation (Dadachova *et al.*, 2007).

By fixation of the meristematic growth of small populations, these can recurrently separate from the ancestral, phenotypically plastic, generalist species. The asexual *Exophiala dermatitidis*, for example, can transform into a meristematic form at ultralow pH (de Hoog *et al.*, 1994). However, several stress-tolerant strains of this species are known to irreversibly maintain their slowly expanding, meristematic morphology. The same has been noted even more frequently for the related species *Exophiala phaeomuriformis* (Matsumoto *et al.*, 1986). Meristematic and yeast-like strains do not differ in variable loci of their rDNA (Matos *et al.*, 2003). In other comparable cases, some sequence divergence has been seen, which possibly indicates incipient speciation (reviewed in Selbmann *et al.*, 2005). This genetic fixation and possible subsequent speciation occur in geographical sympatry. However, this type of speciation is seen repeatedly in only a few fungal orders, such as *Capnodiales*, suggesting that drift leads to specific adaptation only if some extremotolerance is already present (Selbmann *et al.*, 2005).

The remarkable plasticity of some groups of species can be partially attributed to cryptic genetic variation that is not detected by sequence analysis of typical marker genes (Schlichting, 2008). Genetic variation can remain undetected in unstudied regulatory parts of analysed genes, and it is likely to be expressed when an organism encounters a rare or a novel environment (Schlichting, 2008). Furthermore, gene copy number variations in populations appear to contribute to the ecological fitting of fungi (Corradi & Sanders, 2006). Fixation of gene copy numbers can then lead to ecological specialization of lineages. These variations can leave corresponding signatures in the genomes. Soanes *et al.* (2008) recently showed that plant pathogenic fungi have characteristic gene-family expansions. These interesting findings of comparative genome analysis suggest that certain ecological signatures can be found in the genomes of ecologically distinct fungi. This particularly appears to affect genes that are involved in stress response. Wapinski *et al.* (2007) have shown that stress-related genes have many duplications and losses, whereas growth-related genes show selection against such changes. Interestingly, almost every stress-responsive gene analysed in the extremely halotolerant *H. werneckii* is present in at least two copies (Gorjan & Plemenitaš, 2006; Lenassi & Plemenitaš, 2007; Vaupotič & Plemenitaš, 2007a; Vaupotič *et al.*, 2007; Gostinčar *et al.*, 2009).

Examples of the roles of isolated populations in the first stage of adaptation have been discovered in glacial populations of *P. crustosum* (Sonjak *et al.*, 2007a) and *A. pullulans* (Zalar *et al.*, 2008b), as described above. The characteristic of a strong founder effect has been seen in both cases: the

glacial populations were genetically homogenous and differed from populations in more moderate environments at the molecular as well as at the morphological/physiological level. Genetic divergence in *P. crustosum* was limited and founder populations did not form distinct lineages. In contrast, *A. pullulans* populations displayed significant differences and a concordant variation at various loci. The low divergence of *P. crustosum* in the few studied loci, nevertheless, does not imply low diversification at a genomic scale or at physiological level. One of the glacial populations of *P. crustosum* showed an unusual inability to grow on creatine as the sole source of carbon, a trait that is strikingly similar to antagonistic pleiotropy. This latter phenomenon was also recognized in an experimental evolution approach as an inability to grow on a wide range of carbon sources (MacLean, 2005). In initial growth phases, the colonies of glacial *A. pullulans* are more translucent, with slow-growing cells that are smaller than in isolates from other sources. These characteristics easily revert after several plate transfers, indicating a plastic response to the glacial environment with considerable maintenance over generations (unpublished data). Epigenetic phenomena that have been described recently as a form of stress response in plants might be responsible for this phenomenon (Molinier *et al.*, 2006), but have not yet been described in fungi.

As the environment at the ecological extremes is stressful by definition, an increase in mutation rates as a stress response can play a fundamental role in adaptation to new conditions. This response has been seen in a variety of organisms, in the laboratory as well as in the wild strains of, for example, *Escherichia coli* (Rosenberg *et al.*, 1998; Bjedov *et al.*, 2003; Hastings *et al.*, 2004), *S. cerevisiae* (Heidenreich *et al.*, 2003) and *Caenorhabditis elegans* (Rosenberg & Hastings, 2004). Freezing can increase the mutation rates of mtDNA in *S. cerevisiae* (Stoycheva *et al.*, 2007), and even activate retrotransposons (Stamenova *et al.*, 2008). The inherited spontaneous mutation rates in *Penicillium lanosum* and *Aspergillus niger* correlate to the levels of stress in the environment they were isolated from (Lamb *et al.*, 2008). These data suggest that the mutation rate is an important and adaptable feature.

Adaptive radiation

If conditions at the ecological periphery of a species are stable, the above processes will potentially lead to genetic divergence of their respective populations. It is therefore interesting that most extremotolerant fungi do not produce fertile, meiotic structures and can diverge as asexuals. Thus, divergence is enforced by the cessation of gene flow from other subpopulations.

This speciation might have occurred in the genus *Thelebolus*, as described above (de Hoog *et al.*, 2005a). In hypersaline environments, no less than seven new *C. sphaerospermum*-like

species have been found (Zalar *et al.*, 2007), among several other new species (Zalar *et al.*, 1999a, b, 2008a; Butinar *et al.*, 2005b). The abundance of emerging and specialized species in stress-tolerant groups might be the result of colonization and pronounced persistence in the isolated peripheral environments, until the dispersal of genetically adapted strains becomes effective. Divergence of species is characteristic of the initial phase of adaptive radiation (Gavrilets & Losos, 2009). We think that diversification at this stage is significant even when the fungus only maintains asexual propagation. Mitotic recombination in asexual species can accelerate adaptation and drive diversification, as shown by Schoustra *et al.* (2007). Some models, however, show that small peripheral populations can indeed benefit from a moderate gene influx, as it opposes the detrimental effects of genetic drift and increases the probability of the introduction of fit alleles from the core population (Alleaume-Benharira *et al.*, 2006). Several studies have revealed strong positive correlations of diversity and stress under conditions near the edge of life, and it has been suggested that increases in the mutation rate, recombination and gene conversion ensure higher levels of genetic diversity, providing greater potential for genetic adaptation (Nevo, 2001; Kis-Papo *et al.*, 2003). However, at the extreme limits of life, genotypic diversity declines (Kis-Papo *et al.*, 2003).

Specialization

Further specialization by purifying selection can shift growth optima from the ancestral ecological amplitude towards the extremes of the ancestral ranges. In the evolutionary course, the ancestral species can become extinct or can accumulate enough differences such that it is not regarded as being closely related anymore.

The evolutionary fate of specialists is not clear. Emerging empirical evidence suggests that specialist species of plants and animals are declining throughout the World, due to habitat loss (reviewed in Devictor *et al.*, 2008). Specialists are expected to be negatively affected by landscape disturbance, as natural selection has favoured their development in stable environments. To date, no such data exist at the level of microorganisms. However, it is possible that they escape the detrimental conditions more easily than organisms with a more complex organization (such as plants) by switching their biotopes (e.g. substrates or hosts) to maintain physicochemically similar microecological situations. This recalls the law of relative habitat constancy, originally described in plant ecology (Walter & Walter, 1953). For example, diverse black fungi commonly colonize lichens in dry habitats (Harutyunyan *et al.*, 2008). Also, veins of liquid water that perforate glacial ice can contain significant concentrations of ions expelled during ice formation (Price, 2000), while the freezing itself reduces the water activity (Price, 2009), thus making glacier veins, in this way, similar

to hypersaline environments. Indeed, studies have shown significant overlap of fungal diversity between both environments (Gunde-Cimerman *et al.*, 2003). One of the species that inhabits both salterns and glaciers is *Cladosporium halotolerans* (Zalar *et al.*, 2007), which is also found in osmotically similarly stressful habitats in bathrooms (unpublished data). Associated extremophilic bacterial communities as a selective force could also affect the phenotype of fungi (e.g. Schroeckh *et al.*, 2009). These biotope switches are perhaps of great, and so far poorly explored, importance in the evolution of microorganisms. We might discover new close relatives of apparently isolated and specialized microorganisms by screening uncommon habitats. This might well be the case for the very distinct lineage of the enigmatic and fundamentally extremophilic *W. ichthyophaga*. Imaginative environmental sampling should shed additional light on the evolution of extremotolerant fungi.

Other possible examples of biotope switching and habitat constancy could be envisioned in the context of the emerging pathogens. The role of unexpected reservoirs of opportunistic human pathogens among bacteria has been highlighted recently (Berg *et al.*, 2005). Although we rarely think about it as such, the warm-blooded animal body and skin represent an extreme environment for microorganisms, with elevated temperatures and salt levels, as well as mechanic stress. A pathogen must also successfully counteract the host immune responses, as well as other defence strategies. It has already been proposed that the human pathogenicity of fungi is associated with moderate osmotolerance at the order level (de Hoog *et al.*, 2005b). Generalistic fungal groups with suitable preadaptation or exaptation might therefore represent a pool of potential medically important fungi, which might readily switch from environmental niches to human bodies (e.g. Badali *et al.*, 2008; Li *et al.*, 2008).

Conclusions

We have proposed an order of events that can explain the adaptive processes that allow life in environments hostile to most organisms. Understanding the evolution of microorganisms in extreme environments will increase our basic knowledge of evolutionary processes and allow a better evaluation of the potential ecological consequences of environmental changes and human health.

If, as we believe, extreme environments are important for evolutionary processes, human activities may influence biodiversity even more than previously thought. Peripheral environments, from Arctic glaciers to deserts, are those that are most influenced by climate changes. Their populations are more vulnerable, due to their small size and fragmentation. After millennia of microbial evolution, the stability of these fragile environments is threatened. The consequences of this disruption are still unpredictable.

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References

- Agosta SJ & Klemens JA (2008) Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. *Ecol Lett* **11**: 1123–1134.
- Aihara M, Tanaka T & Takatori K (2001) *Cladosporium* as the main fungal contaminant of locations in dwelling environments. *Biocontrol Sci* **6**: 49–52.
- Alleaume-Benharira M, Pen IR & Ronce O (2006) Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *J Evolution Biol* **19**: 203–215.
- Andrews JH, Spear RN & Nordheim EV (2002) Population biology of *Aureobasidium pullulans* on apple leaf surfaces. *Can J Microbiol* **48**: 500–513.
- Badali H, Gueidan C, Najafzadeh MJ, Bonifaz A, van den Ende AHGG & de Hoog GS (2008) Biodiversity of the genus *Cladophialophora*. *Stud Mycol* **61**: 175–191.
- Badillet G, de Bièvre C & Spizajzen S (1982) Isolement de dématiées à partir d'ongles et de squames. *B Soc Fr Mycol* **11**: 69–72.
- Berg G, Eberl L & Hartmann A (2005) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ Microbiol* **7**: 1673–1685.
- Bermejo JM, Dominguez JB, Goni FM & Uruburu F (1981) Influence of pH on the transition from yeast-like cells to chlamydospores in *Aureobasidium pullulans*. *Antonie van Leeuwenhoek* **47**: 385–392.
- Bjedov I, Tenaillon O, Gerard B, Souza V, Denamur E, Radman M, Taddei F & Matic I (2003) Stress-induced mutagenesis in bacteria. *Science* **300**: 1404–1409.
- Bridle JR & Vines TH (2007) Limits to evolution at range margins: when and why does adaptation fail? *Trends Ecol Evol* **22**: 140–147.
- Buckling A, Wills MA & Colegrave N (2003) Adaptation limits diversification of experimental bacterial populations. *Science* **302**: 2107–2109.
- Butinar L, Sonjak S, Zalar P, Plemenitaš A & Gunde-Cimerman N (2005a) Melanized halophilic fungi are eukaryotic members of microbial communities in hypersaline waters of solar salterns. *Bot Mar* **48**: 73–79.
- Butinar L, Zalar P, Frisvad JC & Gunde-Cimerman N (2005b) The genus *Eurotium* – members of indigenous fungal community in hypersaline waters of salterns. *FEMS Microbiol Ecol* **51**: 155–166.
- Christner BC, Mosley-Thompson E, Thompson LG, Zagorodnov V, Sandman K & Reeve JN (2000) Recovery and identification of viable bacteria immured in glacial ice. *Icarus* **144**: 479–485.

- Colakoglu G (2003) Airborne fungal spores at the Belgrad forest near the city of Istanbul (Turkey) in the year 2001 and their relation to allergic diseases. *J Basic Microb* **43**: 376–384.
- Corradi N & Sanders IR (2006) Evolution of the P-type II ATPase gene family in the fungi and presence of structural genomic changes among isolates of *Glomus intraradices*. *BMC Evol Biol* **6**: 21.
- Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD & Casadevall A (2007) Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. *PLoS One* **2**: e457.
- de Boer W, de Ridder-Duine AS, Klein Gunnewiek PJA, Smant W & Van Veen JA (2008) Rhizosphere bacteria from sites with higher fungal densities exhibit greater levels of potential antifungal properties. *Soil Biol Biochem* **40**: 1542–1544.
- de Hoog GS, Takeo K, Yoshida S, Gottlich E, Nishimura K & Miyaji M (1994) Pleoanamorphic life cycle of *Exophiala* (*Wangiella*) *dermatitidis*. *Antonie van Leeuwenhoek* **65**: 143–153.
- de Hoog GS, Göttlich E, Platas G, Genilloud O, Leotta G & van Brummelen J (2005a) Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Stud Mycol* **51**: 33–76.
- de Hoog GS, Zalar P, Gerrits van den Ende AHG & Gunde-Cimerman N (2005b) Relation of halotolerance to human-pathogenicity in the fungal tree of life: an overview of ecology and evolution under stress. *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya* (Gunde-Cimerman N, Oren A & Plemenitaš A, eds), pp. 371–395. Springer, Dordrecht, the Netherlands.
- Devictor V, Julliard R & Jiguet F (2008) Distribution of specialist and generalist species along spatial gradients of habitat disturbance and fragmentation. *Oikos* **117**: 507–514.
- Gavrilets S & Losos JB (2009) Adaptive radiation: contrasting theory with data. *Science* **323**: 732–737.
- Gorbushina AA, Kotlova ER & Sherstneva OA (2008) Cellular responses of microcolonial rock fungi to long-term desiccation and subsequent rehydration. *Stud Mycol* **61**: 91–97.
- Gorjan A & Plemenitas A (2006) Identification and characterization of ENA ATPases HwENA1 and HwENA2 from the halophilic black yeast *Hortaea werneckii*. *FEMS Microbiol Lett* **265**: 41–50.
- Gostinčar C, Turk M, Trbuha T, Vaupotič T, Plemenitaš A & Gunde-Cimerman N (2008) Expression of fatty-acid-modifying enzymes in halotolerant black yeast *Aureobasidium pullulans* (de Bary) G. Arnaud under salt stress. *Stud Mycol* **61**: 51–59.
- Gostinčar C, Turk M, Plemenitaš A & Gunde-Cimerman N (2009) The expressions of D^9 -, D^{12} -desaturases and an elongase by the extremely halotolerant black yeast *Hortaea werneckii* are salt dependent. *FEMS Yeast Res* **9**: 247–256.
- Gunde-Cimerman N, Zalar P, de Hoog S & Plemenitaš A (2000) Hypersaline waters in salterns – natural ecological niches for halophilic black yeasts. *FEMS Microbiol Ecol* **32**: 235–240.
- Gunde-Cimerman N, Sonjak S, Zalar P, Frisvad JC, Diderichsen B & Plemenitaš A (2003) Extremophilic fungi in Arctic ice: a relationship between adaptation to low temperature and water activity. *Phys Chem Earth* **28**: 1273–1278.
- Gunde-Cimerman N, Frisvad JC, Zalar P & Plemenitas A (2005a) Halotolerant and halophilic fungi. *Biodiversity of Fungi: Their Role in Human Life* (Deshmukh SK & Rai MK, eds), pp. 69–127. Oxford & IBH Publishing Co. Pvt. Ltd, New Delhi, India.
- Gunde-Cimerman N, Oren A & Plemenitaš A (2005b) *Adaptation to Life in High Salt Concentrations in Archaea, Bacteria, and Eukarya*. Springer, Dordrecht, the Netherlands.
- Harutyunyan S, Muggia L & Grube M (2008) Black fungi in lichens from seasonally arid habitats. *Stud Mycol* **61**: 83–90.
- Hastings PJ, Slack A, Petrosino JF & Rosenberg SM (2004) Adaptive amplification and point mutation are independent mechanisms: evidence for various stress-inducible mutation mechanisms. *PLoS Biol* **2**: e399.
- Heidenreich E, Novotny R, Kneidinger B, Holzmann V & Wintersberger U (2003) Non-homologous end joining as an important mutagenic process in cell cycle-arrested cells. *EMBO J* **22**: 2274–2283.
- Hocking AD, Miscamble B & Pitt J (1994) Water relations of *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Curvularia lunata* and *Curvularia pallescens*. *Mycol Res* **98**: 91–94.
- Hölker U, Bend J, Pracht R, Tetsch L, Müller T, Hofer M & de Hoog GS (2004) *Hortaea acidiphila*, a new acid-tolerant black yeast from lignite. *Antonie van Leeuwenhoek* **86**: 287–294.
- Johannesson K & Andre C (2006) Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Mol Ecol* **15**: 2013–2029.
- Katz Ezov T, Boger-Nadjar E, Frenkel Z, Katsperovski I, Kemeny S, Nevo E, Korol A & Kashi Y (2006) Molecular-genetic biodiversity in a natural population of the yeast *Saccharomyces cerevisiae* from 'Evolution Canyon': microsatellite polymorphism, ploidy and controversial sexual status. *Genetics* **174**: 1455–1468.
- Kim J-H, Kim M-R, Lee J-H, Lee J-W & Kim S-K (2000) Production of high molecular weight pullulan by *Aureobasidium pullulans* using glucosamine. *Biotechnol Lett* **22**: 987–990.
- Kis-Papo T, Kirzhner V, Wasser SP & Nevo E (2003) Evolution of genomic diversity and sex at extreme environments: fungal life under hypersaline Dead Sea stress. *P Natl Acad Sci USA* **100**: 14970–14975.
- Kogej T, Wheeler MH, Lanisnik Rizner T & Gunde-Cimerman N (2004) Evidence for 1,8-dihydroxynaphthalene melanin in three halophilic black yeasts grown under saline and non-saline conditions. *FEMS Microbiol Lett* **232**: 203–209.
- Kogej T, Ramos J, Plemenitas A & Gunde-Cimerman N (2005) The halophilic fungus *Hortaea werneckii* and the halotolerant fungus *Aureobasidium pullulans* maintain low intracellular cation concentrations in hypersaline environments. *Appl Environ Microb* **71**: 6600–6605.
- Kogej T, Gostinčar C, Volkmann M, Gorbushina AA & Gunde-Cimerman N (2006) Mycosporines in extremophilic

- fungi – novel complementary osmolytes? *Environ Chem* **3**: 105–110.
- Kogej T, Stein M, Volkmann M, Gorbushina AA, Galinski EA & Gunde-Cimerman N (2007) Osmotic adaptation of the halophilic fungus *Hortaea werneckii*: role of osmolytes and melanization. *Microbiology* **153**: 4261–4273.
- Koufopanou V, Hughes J, Bell G & Burt A (2006) The spatial scale of genetic differentiation in a model organism: the wild yeast *Saccharomyces paradoxus*. *Philos T Roy Soc B* **361**: 1941–1946.
- Kvitek DJ, Will JL & Gasch AP (2008) Variations in stress sensitivity and genomic expression in diverse *Saccharomyces cerevisiae* isolates. *PLoS Genet* **4**: e1000223.
- Lamb BC, Mandaokar S, Bahsoun B, Grishkan I & Nevo E (2008) Differences in spontaneous mutation frequencies as a function of environmental stress in soil fungi at 'Evolution Canyon,' Israel. *P Natl Acad Sci USA* **105**: 5792–5796.
- Leathers TD (2003) Biotechnological production and applications of pullulan. *Appl Microbiol Biot* **62**: 468–473.
- Lenassi M & Plemenitaš A (2007) Novel group VII histidine kinase HwHhk7B from the halophilic fungi *Hortaea werneckii* has a putative role in osmosensing. *Curr Genet* **51**: 393–405.
- Li DM, de Hoog GS, Saunte DML, van den Ende AHGG & Chen XR (2008) *Coniosporium epidermidis* sp. nov., a new species from human skin. *Stud Mycol* **61**: 131–136.
- Lugauskas A, Sveistyte L & Ulevicius V (2003) Concentration and species diversity of airborne fungi near busy streets in Lithuanian urban areas. *Ann Agr Env Med* **10**: 233–239.
- Ma LJ, Rogers SO, Catranis CM & Starmer WT (2000) Detection and characterization of ancient fungi entrapped in glacial ice. *Mycologia* **92**: 286–295.
- MacLean RC (2005) Adaptive radiation in microbial microcosms. *J Evolution Biol* **18**: 1376–1386.
- Matheny PB, Gossmann JA, Zalar P, Kumar TKA & Hibbett DS (2006) Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. *Can J Botany* **84**: 1794–1805.
- Matos T, Haase G, Gerrits van den Ende AH & de Hoog GS (2003) Molecular diversity of oligotrophic and neurotrophic members of the black yeast genus *Exophiala*, with accent on *E. dermatitidis*. *Antonie van Leeuwenhoek* **83**: 293–303.
- Matsumoto T, Padhye AA, Ajello L & McGinnis MR (1986) *Sarcinomyces phaeomuriformis*: a new dematiaceous hyphomycete. *J Med Vet Mycol* **24**: 395–400.
- Molinier J, Ries G, Zipfel C & Hohn B (2006) Transgeneration memory of stress in plants. *Nature* **442**: 1046–1049.
- Nevo E (2001) Evolution of genome-phenome diversity under environmental stress. *P Natl Acad Sci USA* **98**: 6233–6240.
- Onofri S (1999) Antarctic microfungi. *Enigmatic Microorganisms and Life in Extreme Environments* (Seckbach J, ed), pp. 323–336. Kluwer Academic, Dordrecht, the Netherlands.
- Oren A (1999) Bioenergetic aspects of halophilism. *Microbiol Mol Biol R* **63**: 334–348.
- Park HG, Managbanag JR, Stamenova EK & Jong SC (2004) Comparative analysis of common indoor *Cladosporium* species based on molecular data and conidial characters. *Mycotaxon* **89**: 441–451.
- Pereira PT, de Carvalho MM, Girio FM, Roseiro JC & Amaral-Collaco MT (2002) Diversity of microfungi in the phylloplane of plants growing in a Mediterranean ecosystem. *J Basic Microb* **42**: 396–407.
- Petrovič U, Gunde-Cimerman N & Plemenitaš A (2002) Cellular responses to environmental salinity in the halophilic black yeast *Hortaea werneckii*. *Mol Microbiol* **45**: 665–672.
- Plemenitaš A, Vaupotic T, Lenassi M, Kogej T & Gunde-Cimerman N (2008) Adaptation of extremely halotolerant black yeast *Hortaea werneckii* to increased osmolarity: a molecular perspective at a glance. *Stud Mycol* **61**: 67–75.
- Price PB (2000) A habitat for psychrophiles in deep Antarctic ice. *P Natl Acad Sci USA* **97**: 1247–1251.
- Price PB (2009) Microbial genesis, life and death in glacial ice. *Can J Microbiol* **55**: 1–11.
- Price PB & Sowers T (2004) Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *P Natl Acad Sci USA* **101**: 4631–4636.
- Ranta HM (1990) Effect of simulated acid rain on quantity of epiphytic microfungi on Scots pine (*Pinus sylvestris* L.) needles. *Environ Pollut* **67**: 349–359.
- Rohde RA & Price PB (2007) Diffusion-controlled metabolism for long-term survival of single isolated microorganisms trapped within ice crystals. *P Natl Acad Sci USA* **104**: 16592–16597.
- Rosenberg SM & Hastings PJ (2004) Genomes: worming into genetic instability. *Nature* **430**: 625–626.
- Rosenberg SM, Thulin C & Harris RS (1998) Transient and heritable mutators in adaptive evolution in the lab and in nature. *Genetics* **148**: 1559–1566.
- Schlichting CD (2008) Hidden reaction norms, cryptic genetic variation, and evolvability. *Ann NY Acad Sci* **1133**: 187–203.
- Schoustra SE, Debets AJ, Slakhorst M & Hoekstra RF (2007) Mitotic recombination accelerates adaptation in the fungus *Aspergillus nidulans*. *PLoS Genet* **3**: e68.
- Schroeckh V, Scherlach K, Nützmanna H-W, Shelest E, Schmidt-Heck W, Schuemann J, Martine K, Hertweck C & Brakhage AA (2009) Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *P Natl Acad Sci USA* **106**: 14558–14563.
- Selbmann L, de Hoog GS, Mazzaglia A, Friedmann EI & Onofri S (2005) Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Stud Mycol* **51**: 1–32.
- Shiomi N, Yasuda T, Inoue Y, Kusumoto N, Iwasaki S, Katsuda T & Katoh S (2004) Characteristics of neutralization of acids by newly isolated fungal cells. *J Biosci Bioeng* **97**: 54–58.
- Singh RS, Saini GK & Kennedy JF (2008) Pullulan: microbial sources, production and applications. *Carbohydr Polym* **73**: 515–531.
- Soanes DM, Alam I, Cornell M, Wong HM, Hedeler C, Paton NW, Rattray M, Hubbard SJ, Oliver SG & Talbot NJ (2008) Comparative genome analysis of filamentous fungi reveals

- gene family expansions associated with fungal pathogenesis. *PLoS One* **3**: e2300.
- Sonjak S, Frisvad JC & Gunde-Cimerman N (2007a) Genetic variation among *Penicillium crustosum* isolates from the Arctic and other ecological niches. *Microb Ecol* **54**: 298–305.
- Sonjak S, Ursic V, Frisvad JC & Gunde-Cimerman N (2007b) *Penicillium svalbardense*, a new species from Arctic glacial ice. *Antonie van Leeuwenhoek* **92**: 43–51.
- Sonjak S, Frisvad JC & Gunde-Cimerman N (2009) Fingerprinting using extrolite profiles and physiological data shows sub-specific groupings of *Penicillium crustosum* strains. *Mycol Res* **113**: 836–841.
- Stamenova R, Dimitrov M, Stoycheva T, Pesheva M, Venkov P & Tsvetkov TS (2008) Transposition of *Saccharomyces cerevisiae* Ty1 retrotransposon is activated by improper cryopreservation. *Cryobiology* **56**: 241–247.
- Sterflinger K (1998) Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. *Antonie van Leeuwenhoek* **74**: 271–281.
- Stoycheva T, Venkov P & Tsvetkov T (2007) Mutagenic effect of freezing on mitochondrial DNA of *Saccharomyces cerevisiae*. *Cryobiology* **54**: 243–250.
- Turk M & Plemenitaš A (2002) The HOG pathway in the halophilic black yeast *Hortaea werneckii*: isolation of the HOG1 homologue gene and activation of HwHog1p. *FEMS Microbiol Lett* **216**: 193–199.
- Turk M, Mejanelle L, Šentjurs M, Grimalt JO, Gunde-Cimerman N & Plemenitaš A (2004) Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. *Extremophiles* **8**: 53–61.
- Turk M, Abramovic Z, Plemenitaš A & Gunde-Cimerman N (2007) Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. *FEMS Yeast Res* **7**: 550–557.
- Vadkertiova R & Slavikova E (1995) Killer activity of yeasts isolated from the water environment. *Can J Microbiol* **41**: 759–766.
- Vaupotic T & Plemenitaš A (2007a) Osmoadaptation-dependent activity of microsomal HMG-CoA reductase in the extremely halotolerant black yeast *Hortaea werneckii* is regulated by ubiquitination. *FEBS Lett* **581**: 3391–3395.
- Vaupotic T & Plemenitaš A (2007b) Differential gene expression and Hog1 interaction with osmoresponsive genes in the extremely halotolerant black yeast *Hortaea werneckii*. *BMC Genomics* **8**: 280–295.
- Vaupotic T, Gunde-Cimerman N & Plemenitaš A (2007) Novel 3'-phosphoadenosine-5'-phosphatases from extremely halotolerant *Hortaea werneckii* reveal insight into molecular determinants of salt tolerance of black yeasts. *Fungal Genet Biol* **44**: 1109–1122.
- Walter H & Walter E (1953) Einige allgemeine Ergebnisse unserer Forschungsreise nach Südwestafrika 1952/53: Das Gesetz der relativen Standortskonstanz; das Wesen der Pflanzengemeinschaften. *Ber Deut Bot Ges* **66**: 228–236.
- Wapinski I, Pfeffer A, Friedman N & Regev A (2007) Natural history and evolutionary principles of gene duplication in fungi. *Nature* **449**: 54–61.
- Webb JS, Van der Mei HC, Nixon M, Eastwood IM, Greenhalgh M, Read SJ, Robson GD & Handley PS (1999) Plasticizers increase adhesion of the detriogenic fungus *Aureobasidium pullulans* to polyvinyl chloride. *Appl Environ Microb* **65**: 3575–3581.
- West-Eberhard MJ (2005) Developmental plasticity and the origin of species differences. *P Natl Acad Sci USA* **102**: 6543–6549.
- Wicklow D & Malloch D (1971) Studies in the genus *Thelebolus* temperature optima for growth and ascocarp development. *Mycologia* **63**: 118–131.
- Zalar P, de Hoog GS & Gunde-Cimerman N (1999a) *Trimmatostroma salinum*, a new species from hypersaline water. *Stud Mycol* **43**: 57–62.
- Zalar P, de Hoog GS & Gunde-Cimerman N (1999b) Taxonomy of the endoconidial black yeast genera *Phaeotheca* and *Hyphospora*. *Stud Mycol* **43**: 49–56.
- Zalar P, de Hoog GS, Schroers HJ, Frank JM & Gunde-Cimerman N (2005) Taxonomy and phylogeny of the xerophilic genus *Wallemia* (*Wallemiomycetes* and *Wallemiales*, cl. et ord. nov.). *Antonie van Leeuwenhoek* **87**: 311–328.
- Zalar P, de Hoog GS, Schroers HJ, Crous PW, Groenewald JZ & Gunde-Cimerman N (2007) Phylogeny and ecology of the ubiquitous saprobe *Cladosporium sphaerospermum*, with descriptions of seven new species from hypersaline environments. *Stud Mycol* **58**: 157–183.
- Zalar P, Frisvad JC, Gunde-Cimerman N, Varga J & Samson RA (2008a) Four new species of *Emericella* from the Mediterranean region of Europe. *Mycologia* **100**: 779–795.
- Zalar P, Gostinčar C, de Hoog GS, Uršič V, Sudhadham M & Gunde-Cimerman N (2008b) Redefinition of *Aureobasidium pullulans* and its varieties. *Stud Mycol* **61**: 21–38.