Differences in spontaneous mutation frequencies as a function of environmental stress in soil fungi at ''Evolution Canyon,'' Israel

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When various wild strains of *Penicillium lanosum* and *Aspergillus niger* were placed in the same mild laboratory environment, their frequencies of new spontaneous mutations were clearly related to whether they had been isolated from a region of high or low microclimatic stress. In the mild environment, the total frequencies of conidial color and morphological mutations in *P. lanosum*, summed over all relevant loci, ranged from 0.29% to 2.4% for six strains from the north-facing, less stressful ''European'' slope (ES/NFS) of ''Evolution Canyon'' I, compared with 6.5–11.6% for five strains from the south-facing ''African'' slope (AS/SFS), which is a much more stressful environment, being harsher, drier, more fluctuating in temperature, and receiving up to eight times more UV radiation than the opposite slope. The corresponding figures for *A. niger* were 0.42–1.50% for three strains from the ES/NFS and 2.3–4.9% for six strains from the AS/SFS. The more mutagenic environment of the AS/SFS than of the ES/NFS means that, in Evolution Canyon, the mutation frequency differences between the very stressful environment and the less stressful environment are probably even larger than the 4- and 6-fold differences found here in a mild laboratory environment. The evidence from these two filamentous fungi, which have no sexual cycle, is that there are inherited differences in spontaneous mutation rates according to the levels of stress in the environment, and this feature may well be adaptive. Evolution Canyon I is at Nahal Oren, Mount Carmel, Israel.

adaptation | *Aspergillus niger* | mutation rates | *Penicillium lanosum*

To what extent do different strains of an organism develop different spontaneous mutation rates, and can those differences be adaptive to different types of environments or situations? Is the spontaneous mutation rate positively correlated with environmental stress, particularly within a species? This is a different issue from whether a particular factor in the wild environment itself induces mutation. One can compare inherent spontaneous mutation rates in different strains of a species, even from contrasting environments, by putting them in a common laboratory environment after selecting for individuals showing no detected mutations. It is important to study more than one species, preferably with different life histories, to see how general any such effect might be.

The ''Evolution Canyon'' model (1–4) provides optimal ecologically sharply divergent microsites for exploring the relation between divergent environmental stress and mutation rates across life from bacteria to mammals sharing the same interslope ecological divergence. The ''African'' south-facing slope (AS/SFS) of Evolution Canyon I receives 200–800% more solar radiation than the ''European'' north-facing slope (ES/NFS) (5). The AS/SFS is harsher, drier, warmer, more fluctuating, and spatially more heterogeneous than the milder, moister, and lusher mesic ES/NFS. Evolution Canyon I is at Nahal Oren, Mount Carmel, Israel. There are ≈100 m at the bottom and 400 m at the top between the slopes of this 100-m-deep canyon, with a 35° dip on the 120-m-long AS/SFS and a 25° dip on the 180-m-long ES/NFS (2).

Stanhope and Daida (6) reviewed evidence showing that environmental stress can cause increased mutation rates either in specific genes or genomewise. They proposed that a mutation-rate strategy that is variable among individuals within a given generation can optimize function. For mutation and stress in humans and mammals, see refs. 7 and 8. Further evidence on stress and mutation is still very much needed in natural populations, the ecological theatre of evolution in action.

We found (9) that the frequencies of new spontaneous mutations expressed in a common mild laboratory environment differed between strains of the fungus *Sordaria fimicola* according to the level of stress in the environment from which they had been isolated. Strains isolated from the more stressful south-facing AS/SFS of Evolution Canyon I had higher inherited spontaneous mutation frequencies than those from the less stressful north-facing ES/NFS. That fungus has no asexual spores and reproduces sexually by ascospores. To see whether natural genetic variation for spontaneous mutation frequencies is a more general phenomenon and is subject to stressful environment-related natural selection, we examined spontaneous mutation frequencies in two other filamentous fungi from Evolution Canyon I, *Penicillium lanosum* and *Aspergillus niger*. In contrast to *S. fimicola*, these two species have abundant asexual conidia, but no sexual cycle. *P. lanosum* and *A. niger* are very common worldwide-distributed soil fungi (10) frequently isolated from the soils of Evolution Canyon I (11, 12). These fungi, like *S. fimicola*, are vegetatively haploid.

We recorded mutation frequencies for morphological and conidial color mutations, summed over all loci (an unknown number) affecting each character, giving more general results than from any one locus. Inocula of conidia for the mutation frequency estimates were taken from colonies that looked fully wild type for morphology and conidial color, and that had grown from single haploid, uninucleate wild-type conidia. Any preexisting visible mutations would have been excluded so that only new spontaneous mutations in the mild laboratory environment (malt agar medium at 30°C in white fluorescent light) would be detected, not any induced in Evolution Canyon.

The working hypothesis was that the harsher, more stressful, and diverse AS/SFS environment favored processes increasing genetic and phenotypic variation, namely, mutation and recombination, as also is generally true for genetic polymorphisms (2, 4). The lack of a sexual cycle in these *Penicillium* and *Aspergillus* species meant that recombination could not be assessed, but using different characters (morphological mutants and conidial color mutants, instead of ascospore pigmentation mutations in

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Table 1. *P. lanosum*, spontaneous mutation frequencies in 11 different strains

Slope and strain	Conidial color mutants, %	Morphological mutants, %	Total mutants, %	Total colonies
ES/NFS				
10	0.83	0.34	1.17	9,297
11	1.79	0.49	2.29	5,294
12	0.81	0.81	1.62	7,396
13	0.29	0.00	0.29	339
14	2.09	0.29	2.38	2,726
15	0.28	0.21	0.49	1,427
AS/SFS				
16	2.83	3.68	6.51	6,742
17	2.79	7.31	10.09	5,599
18	5.65	0.88	6.53	1,823
19	7.16	4.40	11.56	9,914
21	4.54	3.59	8.13	8,701

Homogeneity $2 \times n$ χ^2 tests, within slopes in all cases. Conidial color mutants, ES/NFS, $\chi^2 = 70.21$; df, 5; $P < 0.001$; AS/SFS, $\chi^2 = 234.32$; df, 5; $P < 0.001$. Morphological mutants, ES/NFS, $\chi^2 = 234.32$; df, 5; $P < 0.001$; AS/SFS, $\chi^2 = 190.50$; df, 5; $P < 0.001$. Total mutants, ES/NFS, $\chi^2 = 52.18$; df, 5; $P < 0.001$; AS/SFS, $\chi^2 = 158.64$; df, 5; $P < 0.001$.

Table 2. *A. niger*, spontaneous mutation frequencies in nine different strains

Slope and strain	Conidial color mutants, %	Morphological mutants, %	Total mutants, %	Total colonies
ES/NFS				
22	0.82	0.68	1.50	9,106
23	0.36	0.92	1.28	8,148
24	0.33	0.08	0.42	14,467
AS/SFS				
25	0.78	1.74	2.52	13,585
26	1.45	2.51	3.97	11,801
28	0.63	4.12	4.75	3,349
29	0.24	2.06	2.30	5,966
30	2.08	2.81	4.89	10,568
31	1.13	3.31	4.44	5,862

Homogeneity $2 \times n$ χ^2 tests, within slopes in all cases. Conidial color mutants, ES/NFS, $\chi^2 = 31.84$; df, 2; $P < 0.001$; AS/SFS, $\chi^2 = 156.60$; df, 5; $P < 0.001$. Morphological mutants, ES/NFS, $\chi^2 = 90.39$; df, 2; $P < 0.001$; AS/SFS, $\chi^2 = 92.59$; df, 5; $P < 0.001$. Total mutants, ES/NFS, $\chi^2 = 83.12$; df, 2; $P < 0.001$; AS/SFS, $\chi^2 = 150.73$; df, 5; $P < 0.001$.

S. fimicola) for the mutation studies helps to test how generally the working hypothesis is correct.

The Evolution Canyon project is to study, at a microscale, biodiversity evolution (the patterns and dynamics of genes, genomes, populations, species, communities, and ecosystems) across different groups of organisms from bacteria through fungi, plants, and animals. It tries to assess the relative importance of the driving evolutionary forces caused by interslope differential solar radiation, temperature, and drought stresses that result in physically and biotically different habitats and organisms on the opposite slopes (reviewed in refs. 1–4). There are now four Evolution Canyons under active study in Israel (4), with the present results being from Evolution Canyon I in Mount Carmel.

For DNA damage responses in *A. nidulans*, see refs. 13–15. See ref. 16 for the genomics of the Aspergilli, and see ref. 17 for interactions of UV sensitivity and photoreactivation with the type and distribution of ascospore pigmentation in wild-type and mutant strains of *Ascobolus immersus*, *Sordaria brevicollis*, and *S. fimicola*. For *A. niger* genomics, see http://genome.jgi-psf.org/Aspni1/Aspni1.home.html. A map of *A. niger* showed eight linkage groups based on 84 markers (18).

Results

Tables 1 and 2 show the mutation frequency results for 11 different strains of *P. lanosum* and 9 different strains of *A. niger*, respectively, with pooled results from each slope for both organisms shown in Table 3. The most striking results were the clearly higher spontaneous mutation frequencies in the AS/SFS strains than in the ES/NFS strains in both fungi. In *P. lanosum*, they ranged from 6.51% to 11.56% for the AS/SFS strains, compared with only 0.29–2.38% for the ES/NFS strains, with no overlap between ranges. In *A. niger*, the total mutation frequencies in AS/SFS strains ranged from 2.30% to 4.89%, compared with only 0.42–1.50% for ES/NFS strains, again with no overlap between the ranges.

In the pooled values in Table 3, the mutation frequencies for conidial color mutants, morphological mutants, and total mutants were 4.27-, 8.86-, and 5.68-fold higher in AS/SFS strains than in ES/NFS strains for *P. lanosum*, respectively, with corresponding values of 2.44-, 5.36-, and 3.87-fold in *A. niger*. As shown in the statistical analyses using the Wilcoxon rank sum test in Table 3, all of these differences between slopes for *P. lanosum* were significant, with $P = 0.0022$. For *A. niger*, the differences between slopes were significant ($P = 0.0119$) for total mutations and morphological mutants; conidial mutants were nonsignificantly more frequent in AS/NFS strains than in ES/NFS strains. Table 1 shows that there were no overlaps in the mutation frequency ranges of six ES/NFS strains of *P. lanosum* with those of the five AS/SFS strains for conidial color, morphological mutants, or total mutants. Table 2 shows overlaps in *A. niger* only for conidial color mutants.

As well as these striking differences between strains from the two slopes, there also were clear differences in mutation frequencies between strains from the same slope. The mutation frequency data and the homogeneity χ^2 tests in Tables 1 and 2 demonstrate that effect in both fungi. The relative frequencies of conidial color mutants and morphological mutants differed between strains, with a weak positive correlation between those frequencies (correlation coefficient +0.46 for *A. niger*, +0.51 for *P. lanosum*, and +0.43 for the two fungi combined, with only the last value being significantly different from zero at $P = 0.05$ in a one-tailed test).

There was moderate agreement between repeats and replicates. One expects variation between replicates and repeats because mutations could arise at any stage in colony development, from the initial inoculum to conidial development. It seems that most mutations arose late in conidial development because mutations occurring earlier could result in the same type of mutation (e.g., the same colored conidial mutation) occurring with a high frequency in a particular run. That was only observed once, in *A. niger* strain 24, with almost all mutant colonies having the same pleiotropic mutation, with green conidia instead of the wild-type black, and yellow hyphae instead of wild-type white. That run was omitted from these data because the results were atypical, quite different from replicates and repeats.

Discussion

There were much higher mutation frequencies in strains from the AS/SFS (6.51–11.56% in *P. lanosum*; 2.30–4.89% in *A. niger*), compared with those from the ES/NFS (0.29–2.38% in *P. lanosum*; 0.42–1.50% in *A. niger*). The Evolution Canyon I results (9) with *Sordaria fimicola* showed that strains from the AS/SFS had mutation frequencies of 2.3%, 3.5%, and 4.4% for ascospore pigmentation mutants, clearly and consistently higher than those of strains from the ES/NFS, 0.9%, 1.1%, 1.2%, 1.3%, and 1.3%, with an intermediate value of 2.14% from a strain from the valley bottom. Thus, very similar results were found in a fungus with no

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Table 3. Results combined over all strains on a slope, testing for interslope differences in mutation frequency

Slope from which strains were obtained	No. of strains from that slope	Conidial color mutants ± SE, %	Morphological mutants ± SE, %	Total mutants ± SE, %	Total colonies	Wilcoxon rank sum tests for the difference between slopes		
						Conidial color mutation	Morphological mutation	Total of both types
P. lanosum								
ES/NFS	6	1.11 ± 0.31	0.49 ± 0.11	1.60 ± 0.36	26,479	21*	21*	21*
AS/SFS	5	4.74 ± 0.84	4.34 ± 1.03	9.08 ± 1.00	32,779	45*	45*	45*
A. niger								
ES/NFS	3	0.48 ± 0.16	0.47 ± 0.25	0.95 ± 0.33	31,721	11†	6‡	6‡
AS/SFS	6	1.17 ± 0.27	2.52 ± 0.35	3.68 ± 0.46	51,131	34†	39‡	39‡

*, $P = 0.0022$; †, $P = 0.1905$; ‡, $P = 0.0119$.

conidia, reproducing sexually by ascospores (*S. fimicola*) and in two different fungi, *P. lanosum* and *A. niger*, with very different life histories, having no sexual reproduction, but producing abundant asexual conidia. Thus, studies of 28 strains in three fungal genera, with three different types of characteristics (conidial color, morphological appearance, and ascospore pigmentation), have all shown the same results: much more spontaneous mutation (in the common mild laboratory environment) in strains from the more stressful AS/SFS than in ones from the milder ES/NFS. This is much stronger evidence for a general positive correlation between stress levels and mutation frequencies than from any one fungus. For studies on the effects of sexual reproduction versus conidial (asexual) reproduction on fungal adaptation and distribution, see ref. 19.

In addition to the clear interslope differences in spontaneous mutation frequencies when the strains are grown in the same mild environment, there also were strong differences between strains from the same slope for all three fungi. Mutation frequencies are obviously under genetic control in these haploid fungi. In *Sordaria*, strains were isolated from the top, middle, and bottom of each slope and the valley bottom, with mutation frequencies being unrelated to a strain's position on the slope. All of the *A. niger* and *P. lanosum* strains were from the middle of the slopes. Therefore, intraslope variation does not appear to be related to macroecological differences, whereas the interslope variation does appear to be related. The intraslope variation may relate to sunny and shady microniches within a slope, but we did not study these relations in the present work.

The molecular basis of the mutation frequency differences is unknown. It could be due to differences in DNA-repair enzyme and/or replication proofreading efficiencies. There is a possibility of epigenetic effects of environment. The mutations obtained in our mild environment are stable through serial subcultures. If the environment in Evolution Canyon caused different epigenetic changes in fungi from the different slopes, then growing the different strains in a common environment (in Israel before dispatch and in England before and during these experiments) allows many replications and growth periods in common environments in which any epigenetic differences could be lost. As we measured mutation after much hyphal growth and many nuclear divisions involved in producing the hundreds of millions of conidia used in these experiments, any differences in epigenetic effects would have to persist through >30 nuclear divisions in a common environment. Whatever their cause, the very clear differences in mutation frequencies among strains from the different slopes, in three different fungi, strongly suggests that there has been selection for this difference, and that it is adaptive, with higher mutation frequencies enabling a strain to cope with a more stressful and variable environment through rare favorable mutations, whereas strains in a less stressful and less variable environment have a lower mutation frequency, suffering fewer deleterious mutations. Stress and higher mutation frequencies have been linked in other studies (20).

In each slope, there must be a balance between the advantage of favorable mutations and the disadvantage of unfavorable mutations. In the more stressful environment, with difficult growth conditions, a favorable mutation overcoming a big stress constraint could give a big increase in fitness, which, with so many conidia produced, could more than offset fitness reductions from an increase in the low frequency of unfavorable mutations. Such favorable mutations are more likely to occur in strains with higher mutation frequencies, which would then be selected for at the same time.

It is hard to define what a population is for these colonial fungi with asexual reproduction only (*A. niger* and *P. lanosum*), or sexual reproduction in a homothallic fungus with frequent self-fertilization (*S. fimicola*), and vegetative reproduction by hyphal growth and fragmentation in all three fungi. It is not known how much migration there is within or between slopes, but the very clear difference in spontaneous mutation frequencies between slopes in all three fungi suggests that local selective effects are sufficiently strong to overcome any effects of migration/gene flow. Some gene flow could occur by wind acting on conidia or, because these fungi are coprophilous as well as saprophytic, from migration of cattle or goats between slopes.

In Evolution Canyon I, these three fungi often grow underground, but conidia and ascospores get surface exposure. The conidia of *Aspergillus* and *Penicillium* may be transported long distances by wind, but the much larger and heavier ascospores of *Sordaria* normally dehisce only a few centimeters (data not shown). Strains growing on the ES/NFS are usually shaded by shrubs and other plants, but those strains on the AS/SFS are largely less shaded (1, 2). That would make even more extreme the up to eightfold solar radiation exposure difference between the two slopes for exposed parts of these fungi.

The population genetics of these haploid colonial fungi, two of which have no sexual reproduction, must be very different from that of sexually reproducing diploids, in which harmful recessive mutations can be hidden from selection in heterozygotes. Theoretical aspects of the evolution and genetic control of mutation frequencies were covered by refs. 6 and 21–23, but are normally framed for sexually reproducing diploids. For evidence for the adaptive evolution of mutation rates, see ref. 24.

The visible mutations found in the present experiments would have been largely nonadaptive in the natural habitat, such as lighter conidial colors, different hyphal colors, and morphological mutants (e.g., ones lacking conidia or with altered colony size or growth patterns). Various physiological mutations, not tested for here, might well have been favorable in the natural habitat.

Bos, Stam, and van der Veen (25) described the kinetics of UV survival in *Aspergillus*, stating that resting conidia are in G_1 phase

with a single genome. They found that *A. nidulans* has negligible photorepair, but does have dark repair of UV damage. *A. niger*, with black conidia, had much more UV-resistant conidia than green-spored *A. nidulans*; in both species, mutants with reduced conidial color had less UV resistance than wild type.

The hyphae in *A. niger* and *P. lanosum* usually contain millions of nuclei, so one would expect them to be very UV resistant, compared with haploid uninucleate conidia. In hyphae, loss-of-function mutations, which are usually recessive in diploids and heterokaryons, would have little impact, but would be expressed in conidia and in colonies from conidia. Genes with gain-of-function mutations are likely to be rare, of unpredictable dominance, and more likely to be expressed in conidia and colonies from them than in heterokaryons, where they would be very much rarer than wild-type genes. High mutation rates are unlikely to do much harm in established growing or resting hyphae, except for very rare heterokaryon-dominant mutations, and they will be vastly outnumbered by normal genes. Thus, the selection effects on mutation frequencies are most likely to occur at the conidial stage.

There is a theoretical possibility that some colonies observed with mutant phenotypes could have come from a parasexual cycle because that exists in *A. niger* and *P. chrysogenum* (26). For that to happen would require the following sequence of events: Vegetative compatibility and chance fusion would have to form a heterokaryon between a wild-type haploid and a spontaneous mutation for conidial color or for colony morphology, and a vegetative fusion of two unlike nuclei would have to produce a diploid (such fusions in *A. nidulans* have a frequency of 10^{-6} to 10^{-5}) (27). It also could happen by vegetative fusion between a wild-type nucleus and a mutant nucleus in a heterokaryon produced just by mutation, not vegetative fusion between two strains; survival of the diploid in competition with natural haploids; mitotic crossing-over between the heterozygous locus and its centromere (centromere distances in *A. niger* were mainly in the range 10^{-4} to 10^{-2}) (18); appropriate segregation of daughter chromosomes at mitosis to produce a homozygous diploid nucleus; and that nucleus to get into a conidium and then form a colony.

Mutations in these fungi are usually recessive to wild type (18), so a diploid homozygous recessive nucleus would not be expressed in a heterokaryon. That sequence of parasexual events would produce diploid colonies, presumably with larger conidia than the wild-type haploid (28). No major conidial diameter differences were noted between haploids and any mutant colonies in the present work. A given heterozygous vegetative diploid also would repeatedly give the same mutant phenotype from mitotic crossing over, which was not found here. In *Aspergillus* and *Penicillium*, there have been few or no reports of heterokaryons and diploids from nature (27). No sexual cycle has ever been described for *A. niger* (29). Somatic diploids in *A. niger* and *P. chrysogenum* have only been obtained artificially after forcing heterokaryons between homokaryotic strains with different complementary auxotrophic mutations, and such forcing is highly unlikely in nature.

Although high levels of genetic variation in plant pathogenic strains of *A. niger* have been attributed to parasexuality and/or wind-facilitated gene flow (30), there was no direct evidence of parasexual recombination and none of vegetative diploids. Because the production of a mutant phenotype from parasexual recombination from an initially wild-type strain (derived from a haploid uninucleate conidium) requires mutation, followed by a whole series of different events, some of which have very low probabilities, it is highly unlikely to be frequent in relation to mutant phenotypes arising solely by mutation. All of the strains used in these studies had conidial diameters within the published haploid range (31): 4 to 5 μm for *A. niger* and 2.5 to 3 μm for *P. lanosum*. The rare diploid conidia in such fungi usually have about twice the volume of haploid ones (28).

There is a small possibility that the number of loci affecting morphology and conidial color might differ between strains and between slopes, affecting recorded mutation frequencies, but the uniformity of conidial sizes rules out whole genome duplication or vegetative diploidy in any strain. Gene duplication would mean that both copies would need to be mutated before recessive mutations could show, and the mutation frequencies in all strains were fairly high. There is no reason to suppose that gene copy number of major genes differs between different strains living close to each other in the same canyon.

Because we are interested in the relation to stress of genetic variation from all sources, it is relevant to cover recombination as well as mutation. In *Sordaria fimicola*, we found that there had been selection in the natural environment for higher levels of crossing over and gene conversion in strains from the AS/SFS of Evolution Canyon I, compared with strains from the ES/NFS when measured in a common environment (32).

We studied spontaneous mutation under laboratory conditions for strains from the two slopes, showing that they had different tendencies to mutate in that common mild environment. Total mutation frequencies in that mild environment in strains from the AS/SFS were ≈3-fold higher in *S. fimicola*, 3.9-fold higher in *A. niger*, and 5.7-fold higher in *P. lanosum* than in ones from the ES/NFS. Because solar UV radiation and extremes of temperature are well known to be mutagenic and are much stronger on the AS/SFS than on the ES/NFS, it is highly likely that the actual mutation frequencies (spontaneous mutations as in the mild laboratory environment, plus those induced by natural mutagenic factors in Evolution Canyon, such as UV light) in Evolution Canyon I differ by much more than those amounts, especially in those parts of the fungi on or above the ground. If very high mutation rates were not beneficial in stressful environments like the AS/SFS, then one would expect the spontaneous mutation frequencies in a mild environment to be much less in strains from the AS/SFS, compared with those from the ES/NFS, compensating to some extent for the greater frequency of mutations induced by local mutagenic factors, but that was not the case.

In our comparison of genetic polymorphism of allozymes and DNA markers and sequence polymorphism in Evolution Canyon I, we found higher genetic polymorphism on the more stressful AS/SFS in 9 of 14 (64%) model organisms in diverse taxa across life (4). Likewise, we found the higher mutation rates described previously (9) and here, higher gene conversion, crossing-over (32), DNA repair (33, 34), genome size, retrotransposons, and up-regulation in genome-wide gene expression of yeasts under H_2O_2 stress (35) on the more stressful AS/SFS. Clearly, more stressful environments generate higher diversities across the genome. This provides raw materials to activate the evolutionary process. The Evolution Canyon model is a microcosm of life's evolution and demonstrates not only interslope adaptive radiation, but, most impressively, also incipient sympatric speciation in diverse organisms across life, including bacteria, flowering plants, and rodents, making it an ecological equivalent of the Galapagos Islands (4).

Materials and Methods

Isolation of Strains. The fungal strains were isolated at Evolution Canyon I in Lower Nahal Oren, Mount Carmel, Israel, from soil by the dilution plating method (36) from the middle collecting stations on the opposite slopes (station 2 on AS/SFS and station 6 on ES/NFS), ≈90 m above sea level. They were stored at 4°C and only subcultured for each experiment to minimize any selection in culture.

Media. The medium used throughout was 20 g/liter Oxoid malt extract and 12 g/liter Oxoid Agar technical 3 in demineralized water. For assessing *Aspergil-*

lus mutation frequencies, 0.52 g/liter sodium deoxycholate was added to reduce colony mergers.

Methods. With filamentous fungi such as *A. niger* and *P. lanosum*, which have haploid uninucleate conidia, one can select by eye for colonies of single-conidial origin with no visible morphological or conidial color mutations. One can then plate many conidia from them and score the frequency of visible morphological and conidial color mutants that have arisen in the laboratory culture conditions, not in the different strains' original environments.

Before each experiment, conidia from the main stocks were repeatedly streaked on the medium to obtain colonies originating from single haploid uninucleate conidia. These were subcultured individually onto plates of medium to check that there were no visible mutants in the inoculum used in the flasks. The slight differences within species between wild-type strains in conidial and hyphal color were noted to help identify mutants later; they also served as a check for cross-contamination.

Conidia were grown on 35 ml of medium in 100-ml flasks. All growth was at 30°C in incubators illuminated by daylight-type fluorescent tubes. After growth for 5 (*A. niger*) to 7 (*P. lanosum*) days, conidia were suspended in 10 ml of 0.1% (vol/vol) Tween 80 (polysorbate 80), with vigorous shaking with sterile glass beads. After 5 min standing for settling of larger fragments, the conidial suspensions were filtered through Schleicher & Schuell 604 Rundfilter paper (retention size 12–25 μm). The filtrate was centrifuged for 10 min at 2,795 $\times$ *g*. The pellet was resuspended in 10 ml of deionized water and then sonicated for 7 min in a Branson 151 ultrasonic bath to break up chains into single conidia, with microscope monitoring.

For assessing mutation frequencies, suitable dilutions of conidial suspension were spread on plates containing malt agar (*P. lanosum*) or malt agar with sodium deoxycholate (*A. niger*). Plates were coded for scoring so that the strain was unknown to the scorer. Visible color and morphological mutants were scored, together with total colony counts, after 96 to 120 h, and they were further subcultured to check whether the mutant appearance was maintained, which it nearly always was. The wild-type conidia are gray-green-blue in *P. lanosum* and dark brown to black in *A. niger*. Morphological mutants differed from wild type in colony size, growth pattern, surface appearance, or the absence of conidia. The Israeli strains of these fungi have been described (31).

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