DOI: 10.1534/genetics.104.027235

Dikaryons of the Basidiomycete Fungus *Schizophyllum commune*: Evolution in Long-Term Culture

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Manuscript received February 11, 2004
Accepted for publication May 7, 2004

ABSTRACT

The impact of ploidy on adaptation is a central issue in evolutionary biology. While many eukaryotic organisms exist as diploids, with two sets of gametic genomes residing in the same nucleus, most basidiomycete fungi exist as dikaryons in which the two genomes exist in separate nuclei that are physically paired and that divide in a coordinated manner during hyphal extension. To determine if haploid monokaryotic and dikaryotic mycelia adapt to novel environments under natural selection, we serially transferred replicate populations of each ploidy state on minimal medium for 18 months (~13,000 generations). Dikaryotic mycelia responded to selection with increases in growth rate, while haploid monokaryotic mycelia did not. To determine if the haploid components of the dikaryon adapt reciprocally to one another's presence over time, we recovered the intact haploid components of dikaryotic mycelia at different time points (without meiosis) and mated them with nuclei of different evolutionary histories. We found evidence for coadaptation between nuclei in one dikaryotic line, in which a dominant deleterious mutation in one nucleus was followed by a compensatory mutation in the other nucleus; the mutant nuclei that evolved together had the best overall fitness. In other lines, nuclei had equal or higher fitness when paired with nuclei of other histories, indicating a heterozygote advantage. To determine if genetic exchange occurs between the two nuclei of a dikaryon, we developed a 24-locus genotyping system based on single nucleotide polymorphisms to monitor somatic exchange. We observed genetic exchange and recombination between the nuclei of several different dikaryons, resulting in genotypic variation in these mitotic cell lineages.

ADAPTATION is readily observed in artificial populations of viruses, bacteria, and yeasts under natural selection (Elena and Lenski 2003). Because these organisms can be maintained in large populations with short generation times, rare mutations that confer increased fitness may increase rapidly in frequency and approach fixation in succession. Consequently, an increase in mean population fitness may become apparent in a matter of days or weeks in response to selection. One property of well-mixed populations of unicellular organisms is that the physical distance between individuals is not correlated with relatedness by descent; each individual has the opportunity to replicate and disperse. In contrast, filamentous microorganisms, such as many fungi, grow as physically structured colonies in which the distance among growing tips is correlated with relatedness by descent. In these structured systems, cell division and growth occur predominantly at the hyphal tips near the periphery of the colony and dispersal is restricted such that the descendants remain in adjacent areas. In a filamentous colony, the cells in the interior of the colony may be dormant or may replicate only infrequently.

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Although the evolution of well-mixed, unicellular microorganisms has been studied extensively (Elena and Lenski 2003), the evolution of filamentous microorganisms has not. The overall goal of this study was to determine whether spatially structured fungal mycelia show changes in phenotype in response to selection. For these experiments, we focused on the dikaryon, which typifies the vegetative portion of the life cycles of most basidiomycete fungi. In the dikaryon, the two haploid gametic types of nuclei are maintained indefinitely in paired association. In this study, we maintained dikaryons and haploid monokaryons of the basidiomycete fungus *Schizophyllum commune* in a uniform environment on minimal medium with selection for increased growth rate. The effective population sizes of filamentous colonies propagated in these experiments were undoubtedly smaller than those of well-mixed populations of yeasts or bacteria, allowing for the distinct possibility that deleterious mutations could accumulate >13,000 generations of linear growth in addition to those beneficial mutations that are favored by selection.

The object of this study, *S. commune*, is a model organism for transmission genetics, developmental biology, and population genetics (Raper and Miles 1958; Raper 1966, 1988; Simchen 1966) and has also been used in several different quantitative genetic studies of growth rate (Simchen and Jinks 1964; Simchen 1966; Ander-

son and Deppe 1976; Klein *et al.* 1997). Linear growth rate has been shown to be an accurate and reliable measure for comparing the phenotypes of replicate lines. In this study, linear growth rate was employed as a measure of fitness on the basis of the rationale that larger colonies have the potential to produce more fruit bodies and spores than smaller colonies and therefore to contribute more gametes to the gene pool. We are aware that linear growth rate is not necessarily the sole determinant of fitness in nature, where many other factors, including fruiting abundance and timing, spore germination rates, and mating efficiency, undoubtedly contribute to overall fitness. However, linear growth rate is a suitable fitness correlate and is technically easy to measure.

The life cycle of *S. commune* allows comparison of evolutionary change between two contrasting conformations of nuclei: (a) in mycelia consisting of uninucleate cells with genetically uniform haploid nuclei and (b) in mycelia consisting of dikaryotic cells, each of which contains the two haploid gametic nuclear types in paired association. Although the dikaryon resembles a diploid in that two haploid genomes reside in each cell with full opportunity for genetic complementation, the dikaryon differs from a diploid in that the two haploid genomes remain separated in different nuclei. The two component haploid genomes of the dikaryotic mycelium can be recovered without meiosis through protoplast formation and regeneration (Wessels *et al.* 1976). This feature of the dikaryon permitted us to compare the phenotypes and genotypes of haploid nuclei with different histories of evolution in haploid and dikaryotic mycelia and to test for fitness interactions among nuclear genotypes that had evolved in pairs within dikaryons. This approach is not possible for diploid organisms, from which the recovery of intact gametic haploid genotypes is generally not possible because the haploids from meiosis or mitotic reduction (*e.g.*, as in the parasexual cycle) are subject to genetic shuffling.

A potential complication with the long-term culture of dikaryons is the possibility of genetic exchange between the paired haploid nuclei, which has been documented for mating type and a small number of other markers (Papazian 1954; Crowe 1960; Parag 1962; Ellingboe 1964; Frankel 1979). In this study, we used 24 single nucleotide polymorphisms (SNPs) to determine patterns of general somatic exchange.

Initially, isogenic experimental populations of *S. commune* were propagated to address three questions: (i) Do haploid, monokaryotic, and dikaryotic mycelia respond to selection for increased growth rates?, (ii) Do the haploid components of the dikaryon adapt reciprocally to one another's presence over time?, and (iii) Does genetic exchange occur between the two nuclei in the dikaryons?

MATERIALS AND METHODS

Life cycle of *S. commune*: In *S. commune*, single meiospores germinate to produce haploid, monokaryotic mycelia. Two complex mating-type factors control sexual compatibility in the monokaryons and regulate the maintenance of the dikaryotic state (Raper 1966; Casselton and Olesnicky 1998). Fusion of sexually compatible haploid monokaryotic mycelia results in the formation of the dikaryotic mycelium. The hyphae of *S. commune* dikaryons develop clamp connections at each septum (Raper 1966), while the hyphae of monokaryons do not. The dikaryon is the predominant vegetative structure in *S. commune* and most other basidiomycetes. Under appropriate conditions, the dikaryon produces the fruit bodies within which meiosis occurs (Raper 1966). The monokaryotic and dikaryotic mycelia are capable of indefinite growth, allowing for the maintenance and duplication of the genotype of each ploidy state.

Dikaryon-monokaryon matings: A mechanism for nuclear exchange between dikaryons and monokaryons occurs in *S. commune* and is often referred to as di-mon mating or the "Buller phenomenon" (Raper 1966). In di-mon matings, monokaryotic hyphae are dikaryotized by the migration of a compatible nucleus from dikaryotic hyphae. We used di-mon matings here to place a nuclear type with a recessive lethal mutation into association with other nuclear types in dikaryons without allowing the lethal mutation to be expressed in a haploid monokaryon.

Strains and culture conditions: A progenitor dikaryon was formed from mating two sexually compatible single-spore isolates from two different fruit bodies collected near Portland, Ontario, Canada, in May 2000. The progenitor strains and all derivative strains used in this study are available upon request. A dikaryotic culture was established from a single hyphal tip cell. From this dikaryon, the two progenitor haploid genomes were recovered by protoplast formation and regeneration. The two nuclear types of the progenitor dikaryon are designated N1 and N2 throughout. At this stage, the progenitor dikaryon and component haploid monokaryons are assumed to share the same mitochondrial DNA haplotype. In mating between compatible monokaryons, nuclei from one mate show extensive migration into the resident mycelium of the opposing mate and most of the resulting dikaryons have only one of the two original mtDNA types (Smith *et al.* 1990; Specht *et al.* 1992). Any heteroplasmons that occur near the restricted region of hyphal anastomosis where the mated mycelia merge with one another tend to sort out rapidly to pure mtDNA types with vegetative growth (Saville *et al.* 1998).

Next, 12 dikaryotic (designated DIK-1–DIK-12) and 12 haploid monokaryotic lines, 6 of each nuclear type (designated N1-1–N1-6 and N2-1–N2-6), were started in 10-cm-square petri dishes containing a defined minimal medium (MM; Raper and Hoffman 1974) and incubated at 30°. Each culture was serially transferred to a new petri dish every 2 weeks. Two different transfer protocols were used, split between the 12 dikaryotic lines. Under the first protocol, a 2-mm cube of mycelium and agar medium was transferred from the center of the mycelial front to a new dish ("point" inoculation). Under the second protocol, a 2 × 9-mm rectangle of mycelium and agar was transferred from the growing front to a new dish ("strip" inoculation). The rationale for the two different protocols was that the second protocol may have a larger effective population size than the first, resulting in a different evolutionary trajectory. This later appeared not to be the case and the two protocols were subsequently treated as independent replicates of the same experiment. The progenitor dikaryon, the component progenitor haploids, and the experimen-

tal lines at each transfer were archived in duplicate in 1 ml of 15% glycerol at −80°.

Determination of linear growth rates: To determine linear growth rate, the mycelial fronts of colonies growing on minimal medium in the bottom center of 10-cm-square petri dishes were marked on the fourth and seventh day after inoculation. Three radii were drawn from the bottom center of the petri dish and the distance between each marked front was recorded to the nearest 0.5 mm. Linear growth rates were calculated as the mean from the three radii.

Determination of generation times: Measurements of cell division times were taken as described by Niederpruem *et al.* (1971). In each of the progenitor monokaryons, the progenitor dikaryons, and the evolved dikaryons, hyphal tips were observed under a compound microscope at ×400. The time for the hyphal cell to divide was determined by observing the linear extension of a hyphal tip and the formation of a new septum. This observation was made for several cell divisions on a single hyphal tip and on 10 hyphal tips on the colony margin for each isolate tested. The mean time for a cell duplication cycle was ~1 hr in all cultures, including both dikaryons and monokaryons, progenitors and evolved. Although cell division time remained constant, cell size varied in most lines during the experiment. Cell size, however, varied in direct proportion to the rate at which the mycelial front advanced. In all lines, the mycelial front advanced continuously during the entire 18 months; no cultures occupied the entire plate before the end of a 2-week period and then remained stationary for more than a day. Therefore, the total time for the experiment was 18 months or 1.31×10^4 generations.

Inoculation tests for the presence of dikaryotic-produced growth inhibitor: To determine if the asymmetrical growth form of dikaryons is self-inhibitory due to an extracellular growth inhibitor, a single petri dish was inoculated with adjacent hyphal tips from a single genotype for the progenitor isolates and each of the replicate monokaryons and dikaryons at 9 and 18 months. The hyphal tips were inoculated on minimal medium 5 and 10 mm apart and incubated at 30° for 1 week. A zone free of hyphae between the two inocula was scored as an inhibition reaction, as described by Klein *et al.* (1997).

Component haploid isolation: To isolate the component haploids, the dikaryotic cultures were subjected to a protoplast formation and regeneration protocol. The dikaryotic culture was grown for 3 days in a liquid complete yeast medium (CYM; Raper and Hoffman 1974), macerated for 30 sec in a Waring blender, and grown for an additional day with rotary shaking at room temperature. The harvested cells were then treated with 0.3 g lysing enzyme (Sigma, St. Louis) and 0.1 g of Tv cellulase in 10 ml of a sterile protoplast buffer (0.8 M $MgSO_4$ + 0.02 M sodium citrate, final pH 4.1). After an overnight digestion, the protoplasts were filtered through glass wool packed into the bottom 2 cm of a 10-ml syringe and plated onto solid CYM supplemented with 0.6 M sorbitol. Colonies were picked and those without clamp connections, typically 80–90% of the total, were scored for mating type. From the progenitor dikaryon, the two mating types were recovered in equal frequency; in this genetic background, we did not see the asymmetry in recovery of nuclear types noted by Raper (1985) in some dikaryons.

From each dikaryon, the component haploid genomes were isolated at 9 months (transfer 18) and at 18 months (transfer 39) by protoplast formation and regeneration. For experiments examining interactions among evolved haploid genotypes, each newly recovered haploid was mated with sexually compatible haploids with various histories: (a) evolved in the same dikaryon, (b) evolved in other dikaryons, (c) evolved as haploid monokaryons, and (d) recently recovered from the ancestral archive with as little propagation as possible. The growth rates of these new dikaryons on MM were determined exactly as described by Klein *et al.* (1997).

Nuclear DNA loci: Multiple nuclear regions were used for the multilocus genotyping with no prior knowledge of genomic location or function. PCR primers (Table 1) were designed from the National Center for Biotechnology Information expressed sequence tag database (dbEST) using *S. commune* sequences accessioned to GenBank (Guettler *et al.* 2003).

DNA isolation: The accumulation of viscous polysaccharides in the mycelia of *S. commune* often interferes with genomic DNA isolations. To minimize the accumulation of such polysaccharides, cultures were grown in a carbon-limited minimal medium (0.25% glucose) with light shaking at room temperature for 5 days on the rationale that extracellular carbohydrates would be digested and used as a carbon source by the growing fungus. The harvested mycelium was washed four times with distilled water and filtered through Miracloth (Calbiochem, La Jolla, CA). This protocol allows for a DNA to be extracted that is of higher yield and better quality than DNA obtained from mycelium grown on a rich medium (data not shown). Genomic DNA was prepared by the method of Murray and Thompson (1980).

PCR amplification: PCR reactions (20 μl) contained 4.1 μl gdH_2O, 2 μl 10× PCR buffer, 200 μm deoxynucleoside triphosphates, 0.5 μm of each primer, 0.5 units of Amplitaq DNA polymerase (Perkin-Elmer, Norwalk, CT), and 10 μl of a 100-fold dilution of genomic DNA. Amplifications were carried out in a Perkin-Elmer GeneAmp System 9600 or 9700 thermocycler. The initial denaturation was set at 95° for 8 min, followed by 35 cycles of denaturation at 95°, primer annealing from 55° to 60°, and extension for 30 sec, with a 5-min extension at 72° on the final cycle.

DNA sequencing: Amplified DNAs of the component haploid monokaryons and the progenitor dikaryon were used for sequencing. Sequencing for each region was performed on both strands. PCR products were purified using QIAquick PCR purification kit (QIAGEN, Chatsworth, CA). Sequencing reactions were prepared using the ABI PRISM Big Dye terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Perkin-Elmer), according to the manufacturer's instructions. Cycle sequencing was performed in a Perkin-Elmer GeneAmp System 9600. The reactions were analyzed on the ABI PRISM 310 genetic analyzer (PE Applied Biosystems, Perkin-Elmer).

Oligonucleotide hybridization: The sequenced regions from both monokaryons were aligned using Clustal X (Thompson *et al.* 1997). SNPs were identified and used to design allele-specific oligonucleotide probes containing the potential site of mismatch flanked by seven to eight bases on either side (Table 1). The allele-specific oligonucleotide probes indicated the presence or absence of an allele in the amplicon. Southern blots of PCR-amplified regions were prepared using standard techniques (Sambrook *et al.* 1989). Allele-specific oligonucleotides were end-labeled with [γ-^{32}P]ATP (Perkin-Elmer) and hybridized for 2 hr to the Southern blots at up to 5° above the midpoint temperature of the probe according to the protocol of Saville *et al.* (1998). Three washes of 20 sec each with a wash solution consisting of 6× SSC and 0.5% SDS were made at the same temperature as the hybridization. The blots were then exposed to a phosphor screen for 2–10 min and scanned with a Molecular Dynamics (Sunnyvale, CA) STORM PhosphorImager (Amersham Biosciences) using ImageQuant version 5.2 software. The probes were highly specific to their respective target allele, with little or no hybridization to the

TABLE 1

Allele-specific oligonucleotide primers and probes

Locus (accession no.)	Oligonucleotide sequence[b]	Allele[c]	Temperature[d]
BI135391[a]	TCTCTATTGCGAAGGCTGG (amp)	60	
	CTGCTCAGCATATCCTGTGC (amp)	60	
	TTTTGTCACGTTTCG (hyb)	N1	51
	TTTTGTCCCGTTTCG (hyb)	N2	56
BI135403	TACTGTGTCACGCCCTATCG (amp)	60	
	AGGAAACGGGAATGAGATCC (amp)	60	
	TAGACTGGTACTGTGT (hyb)	N1	40
	TAGACTGAGACTGTGT (hyb)	N2	40
BI135412	TCAGCAAGGATGGGCAAAG (amp)	50	
	GGTCGTAAGATCGTCAGGTAGG (amp)	50	
	GGTTGAGCGCGGAGG (hyb)	N1	64
	GGTTGAGTGCGGAGG (hyb)	N2	58
BG447506	TTCAAGTGCCACCGCTCTAC (amp)	50	
	GACTTCGTCACCGACTTTGC (amp)	50	
	CCGCGCCCTGGGTCG (hyb)	N1	73
	CCGCGCCTTGGGTCG (hyb)	N2	70
BG447515	TTGTGCTCTGACAAGACCGG (amp)	60	
	GCAGAGCTCGATGATGATACC (amp)	60	
	TCAAGCCTTTCAACC (hyb)	N1	51
	TCAAGCCCTTCAACC (hyb)	N2	54
BG518456	GCATCTTCCACCGCATTATC (amp)	60	
	ACTACATGGCGAGCCTTTTG (amp)	60	
	ACACCAACGGCTCGC (hyb)	N1	62
	ACACCAATGGCTCGC (hyb)	N2	59
BG518461	AAAGGTGTCGTGCTGGCTTC (amp)	60	
	CGACCAGCTTCTCATTATACGG (amp)	60	
	AATCCTCACGCGACT (hyb)	N1	54
	AATCCTCGCGCGACT (hyb)	N2	60
BG518471	CTTGGTAACATGGGCTACCTC (amp)	50	
	CGACGAAGATCTTCATGTTCG (amp)	50	
	TCAACGAAGACGCTG (hyb)	N1	55
	TCAACGAGGACGCTG (hyb)	N2	58
BG550556	TCTACCCAGATAGACCGTCG (amp)	60	
	GTGCATGATCATAGCAAGCC (amp)	60	
	CTTCGTCTTCCACTC (hyb)	N1	48
	CTTCGTCATCCACTC (hyb)	N2	49
BG550561	GTACATGTGGGGTGTTGCTG (amp)	55	
	TCTCCAGGTCCTTGTTGAGG (amp)	55	
	GGTTGTCGAGCTTCT (hyb)	N1	50
	GGTTGTCAAGCTTCT (hyb)	N2	47
BG673922	CGCGAACCTAATGACTGAGG (amp)	60	
	ATCTCGATGGCCATGATACC (amp)	60	
	CCCCATTCAAGCTGA (hyb)	N1	56
	CCCCATTTAAGCTGA (hyb)	N2	52
BG673924	CGAAGCAGATCAAGGACAGG (amp)	50	
	CGGTCCATCGTTGTTAACG (amp)	50	
	CACCGGGTGCCGGCA (hyb)	N1	73
	CACCGGGCGCCGGCA (hyb)	N2	77
BG673937	ATACTTCTCCTACAGCGCACG (amp)	55	
	CCTGTAGAGGCATGTTCTTCG (amp)	55	
	ACACCTTCGTGAAGA (hyb)	N1	49
	ACACCTTTGTGAAGA (hyb)	N2	45
BG713751	GTTTGACACGGACAACCTCC (amp)	60	
	AGGTCGACGACCTTGATACC (amp)	60	
	CAATGGGTTCGCCAA (hyb)	N1	60
	CAATGGGCTCGCCAA (hyb)	N2	63
BG713752	ACTTCGACTATATGACCGAGGG (amp)	60	

(*continued*)

TABLE 1

(Continued)

Locus (accession no.)	Oligonucleotide sequence[b]	Allele[c]	Temperature[d]
	TTTCTGGCTTCTCGAACACG (amp)	60	
	GGTGAGTAAATCCCT (hyb)	N1	46
	GGTGAGTGAATCCCT (hyb)	N2	50
BG713762	AAGGAGCGCAAGTACAACG (amp)	60	
	GGAGAATGAAGTTGTGGCC (amp)	60	
	TTTACTTCGGCACGT (hyb)	N1	53
	TTTACTTTGGCACGT (hyb)	N2	50
BF942493	ACTTCAACTTCCGCACCAAC (amp)	50	
	CTTCTTGCCCTTCTTCCCTT (amp)	50	
	TCAACGCTGTCTTCC (hyb)	N1	53
	TCAACGCCGTCTTCC (hyb)	N2	59
BF942494	GTACAGCCAGATGCAGACGA (amp)	60	
	GACTTGGGCACCAACGATAC (amp)	60	
	CGAGGTCATCCTTAT (hyb)	N1	47
	CGAGGTCGTCCTTAT (hyb)	N2	51
BG713749	CGATGAGCTTCTCCTCTTCC (amp)	58	
	TGTGTCCAACCAGCTGTACG (amp)	58	
	GGAAGATGCGCAGGA (hyb)	N1	60
	GGAAGACGCGCAGGA (hyb)	N2	63
BG550553	GTCTTGGCTACAAGGCCAAG (amp)	60	
	GTTGACGATCCAGTTGATGC (amp)	60	
	GTGCCACCTATGGCA (hyb)	N1	55
	GTGCCACTTATGGCA (hyb)	N2	53
BI348515	CATATTCCTTCCGSTAACCGG (amp)	60	
	TAGTTGCTGAAGCTCAGGCC (amp)	60	
	GCATTGTCGATGGTC (hyb)	N1	53
	GCATTGTTGATGGTC (hyb)	N2	48
BG518452	TTGTCGGCGGAAGTTACTCT (amp)	60	
	CTTTGCCAATCCCACAAGTT (amp)	60	
	ACATGCTTGGAACGT (hyb)	N1	51
	ACATGCTCGGAACGT (hyb)	N2	55
BQ173870	CACTTCTTGTACGTCGGCAA (amp)	60	
	ATATTCCAGCTCTTTCGCCA (amp)	60	
	ATGCTCGACCACTTC (hyb)	N1	50
	ATGCTCGGCCACTTC (hyb)	N2	57
BQ173865	AACCTGAGGGTCCATCTGTG (amp)	58	
	GACCCAACCGTACATCCAAC (amp)	58	
	CGTACTTGGAATCCA (hyb)	N1	50
	CGTACTCAGAATCCA (hyb)	N2	48

[a] Sequences from Guettler *et al.* (2003).

[b] Primer sequences amplify accessioned locus (amp). Allele-specific oligonucleotide probes (hyb) are given with the SNP underlined.

[c] Allele assigned from mating type of progenitor monokaryon that has specific hybridization to the corresponding oligonucleotide probe. N1 refers to the mating-type A1B1; N2 refers to the mating-type A2B2.

[d] Annealing temperature for amplification and probe hybridization temperature.

alternate allele (see Figure 1). The probes distinguished each of the two progenitor haploids for 24 SNPs; the progenitor dikaryon was heteroallelic for all 24 SNPs.

RESULTS

Growth rates of haploid and dikaryotic lines: During the 18 months of serial propagation, the haploid and dikaryotic lines showed different patterns of change in linear growth rates (Figures 2–5). In the haploids, the growth rates were initially greater than those of the dikaryons, but did not increase overall during the experiments. In addition, the haploid N2-6 line actually decreased in growth rate. In contrast, some of the dikaryons showed striking increases in growth rates beginning at ~6 months, with high variance in growth rates among the replicate lines. In particular, dikaryons DIK-3 and DIK-8 showed sudden increases in growth rate that persisted for the remainder of the experiment.

Morphology of long-term cultures: Morphological mutations were prevalent in the haploids. In the monokaryons maintained under the "point" transfer protocol,

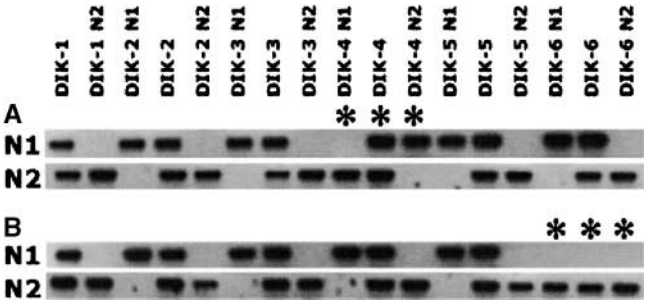

Figure 1.—(A and B) Representative results of the allele-specific multilocus genotyping system. Six dikaryons evolved in culture for 18 months and their recovered haploid components are represented. Asterisks (*) indicate regions of change in genotype. Note that only one haploid was recovered from DIK-1, which harbored a recessive-lethal mutation in one nucleus. (A) An example of reciprocal exchange of alleles at marker locus BG550561 in DIK-4; the expected alleles in the N1 and N2 nuclei are switched relative to the other dikaryons and their haploid components. (B) An example of loss of heteroallelism at marker locus BG673937; only the allele for N2 for marker was found in DIK-6, as shown by the absence of the N1 allele.

the mycelium of N2-1 started as white and fluffy, but suddenly became yellow and appressed to the agar surface at 14 months, a state that persisted throughout the remainder of the experiment. Also, the mycelium of N2-3 suddenly increased in growth rate at 16 months, became appressed to the agar surface, and had leading hyphae that grew in a "corkscrew" pattern. The mutation in N2-3 responsible for the altered morphology is identical in phenotype to the thin (*thn*) mutation that arises frequently and spontaneously in monokaryons of *S. commune* (Raper and Miles 1958). In the monokaryons maintained under the "strip" transfer protocol, the mycelia N1-4, N1-6, and N2-5 all acquired a similar flat, tan morphology with no aerial hyphae at 12, 10, and 10 months, respectively. Finally, N2-6 acquired the same yellow, appressed colony morphology at 8 months as well as that of N2-1 at 17 months. All of the morphological mutations in the monokaryotic lines were recessive. When each of the mutant monokaryons was mated with the progenitor monokaryon of compatible mating type, the colony morphology of the dikaryon was normal. The mating tests also ruled out the possibility that the morphological transitions were the result of contamination. Most of the morphological changes in the monokaryons resulted in altered growth form, but only line N2-4 had a decreased growth associated with the change in colony morphology.

Among the dikaryons, morphological changes of a different nature from those of the monokaryons were associated with an increase in growth rates. The fast-growing mutants arose in the DIK-3 and DIK-8 lines. These dikaryons had a symmetrical colony margin in contrast to the slower-growing progenitor, which had a lobed, asymmetrical colony margin (Figure 6). The asymmetrical growth form of *S. commune* dikaryons collected from nature in a wide geographic range in North America was described by Klein *et al.* (1997). The asymmetrical colony morphology was shown to be light induced; dikaryons grown in complete darkness reverted to a symmetrical, fast-growing colony, similar to the colony morphology and growth rates of the component haploid monokaryons.

Although not tested, Klein *et al.* (1997) hypothesized that the differences in colony morphology (asymmetrical and symmetrical) have a genetic basis and the asymmetrical growth form of dikaryons is self-inhibitory due to an extracellular growth inhibitor. The evidence of the presence of an antagonistic growth inhibitor was seen when the medium in a petri dish was inoculated with adjacent hyphal tips from one single dikaryotic genotype (as in Klein *et al.* 1997). In our experiments, the mycelia of dikaryons with the asymmetrical growth form never grew into contact, yet in the plates inoculated with monokaryons and symmetrically growing dikaryons, the mycelia always grew together without any antagonistic zone (data not shown).

Genetics of mutations in dikaryons: The mutations of large effect for increased growth rate in DIK-3 and DIK-8 were dominant. The evidence for this was that new dikaryons resulting from all pairings of the N2 nuclear type recovered from DIK-3 had a growth rate more than twice that of the progenitor dikaryon, similar to that of DIK-3 at 9 months and beyond (Figure 7). A similar dominant mutation occurred in DIK-8 in the N1 nuclear type. To test if the mutations for fast growth rate in DIK-3 and DIK-8 had an effect in pairings with other nuclei (with genotypes unrelated to the progenitor dikaryon), both were paired with six monokaryons with genotypes unrelated to the progenitor of this experiment. All pairings exhibited the fast growth phenotype (data not shown).

Next, we found that the mutation for increased growth in DIK-3 was due to a single dominant mutation in the N2 nuclear type. Single-spore isolates from DIK-3 at 9 months were paired with their respective compatible mating types from the progenitor to establish progeny dikaryons. The phenotypes of these dikaryons were 46 fast:52 normal, a ratio not significantly different from 1:1. Similarly, progeny from DIK-3 at 18 months segregated 54 fast:45 normal, a ratio again not significantly different from 1:1. We also removed the N2 nuclear type from DIK-3 at 9 and 18 months and paired it with the N1 nuclear type of the progenitor to establish two new dikaryons. On fruiting, single-spore isolates from these two dikaryons were paired with their respective compatible mating types (also from the progenitor) to establish new progeny dikaryons. These dikaryons segregated 48 fast:51 normal (9 months) and 47 fast:53 normal (12 months), ratios that were, once again, not significantly different from 1:1.

In contrast to DIK-3, the fast growth of DIK-8 at 9

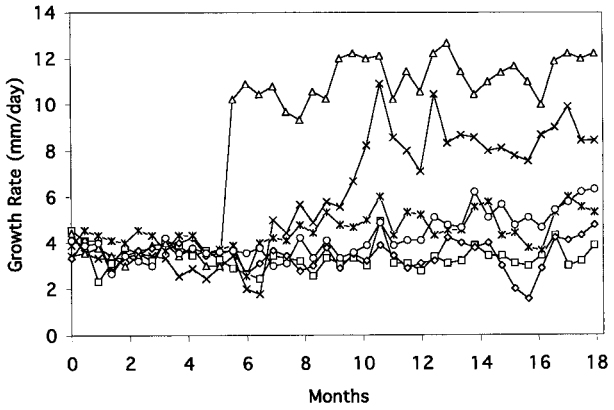

FIGURE 2.—Increase in growth rates of the dikaryotic lines of experiment 1 over 18 months of serial propagation. DIK-1, ×; DIK-2, □; DIK-3, △; DIK-4, ◇; DIK-5, *; DIK-6, ○.

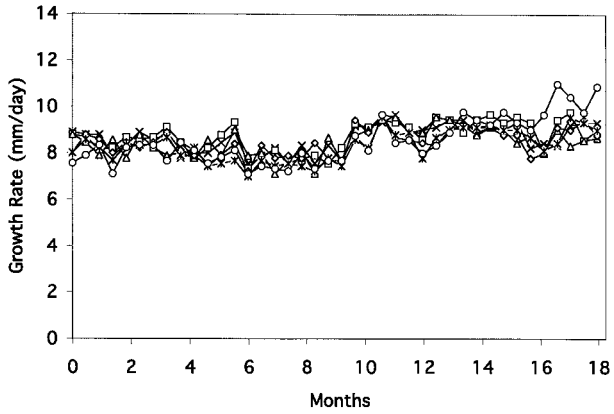

FIGURE 4.—Minimal change in growth rates of the haploid monokaryotic lines of experiment 1 over 18 months of serial propagation. N1-1, ×; N1-2, □; N1-3, △; N2-1, ◇; N2-2, *; N2-3, ○.

and 18 months was not due to a single mutation. DIK-8 at 9 and 18 months was fruited. Single-spore isolates were paired with their respective compatible mating types from the progenitor to establish progeny dikaryons. These dikaryons segregated 26 fast:50 normal:24 slow (9 months) and 24 fast:52 normal:27 slow (18 months), ratios approximating 1:2:1. We also removed the N1 nuclear type from DIK-8 at 9 and 18 months and paired it with the N2 nuclear type of the progenitor to establish two new dikaryons. On fruiting, single-spore isolates from these two dikaryons were paired with their respective compatible mating types (also from the progenitor) to establish new progeny dikaryons. These dikaryons segregated 26 fast:45 normal:24 slow (9 months) and 21 fast:40 normal:19 slow (12 months), ratios that again were not significantly different from 1:2:1. This pattern is consistent with a model that two unlinked genes, *X* and *Y*, are involved. Under this model, the N1 nuclear type in the progenitor is *xy* and has a normal growth rate. A dominant mutation in gene *X* of the N1 type produces slow growth in the dikaryon (*Xy* + *xy*). A compensatory mutation, also in the N1 nuclear type, occurs in the *Y* gene, resulting in a dikaryon with a fast growth rate (*XY* + *xy*). On crossing the N1 nuclear type (*XY*) with the wild type (*xy*), four equally frequent progeny genotypes result: *XY*, *Xy*, *xY*, and *xy*. When placed in dikaryons with a wild-type nucleus, the following phenotypes result: *XY* + *xy*, fast; *Xy* + *xy*, slow; *xY* + *xy*, normal; *xy* + *xy*, normal. We are now in the process of testing this model for fast growth in DIK-8.

To test for possible allelism or gene interaction in the two dominant mutations for fast growth rate, the two nuclei that conferred fast growth rates in DIK-3 and DIK-8 were paired; *i.e.*, DIK-3 N2 was mated with DIK-8 N1. The resulting dikaryon exhibited the fast, symmetrical growth morphology of monokaryotic mycelia, but no fruit bodies or initials were produced after a 1-month incubation and the test for allelism was not possible. In contrast, the mating of the nuclear types of DIK-3 and DIK-8 without the mutations conferring fast growth (*i.e.*, DIK-3 N1 with DIK-8 N2) resulted in dikaryons that had an asymmetrical growth pattern and produced abundant fruit bodies after a 1-week incubation.

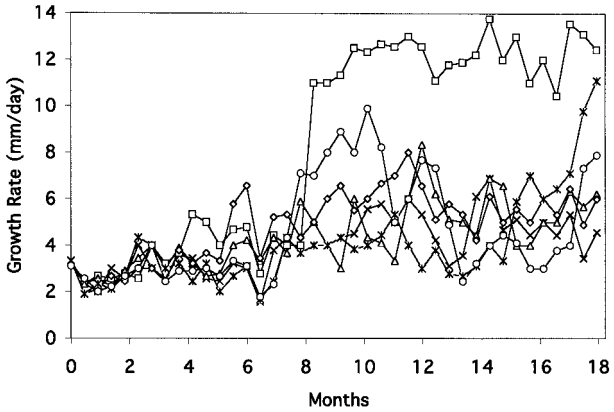

FIGURE 3.—Increase in growth rates of the dikaryotic lines of experiment 2 over 18 months of serial propagation. DIK-7, ×; DIK-8, □; DIK-9, △; DIK-10, ◇ DIK-11, *; DIK-12, ○.

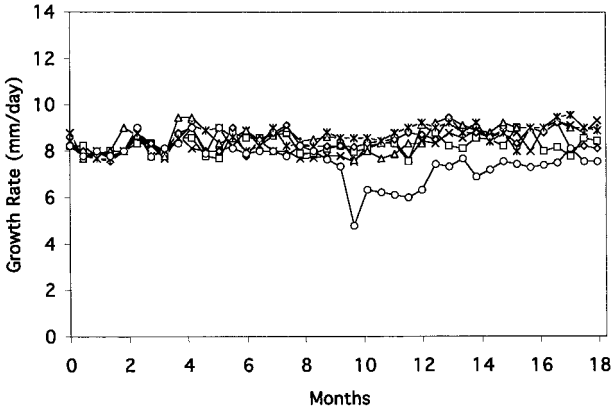

FIGURE 5.—Minimal growth rate change of experiment 2's haploid monokaryotic lines in 18 months of serial propagation. N1-4, ×; N1-5, □; N1-6, △; N2-4, ◇; N2-5, *; N2-6, ○.

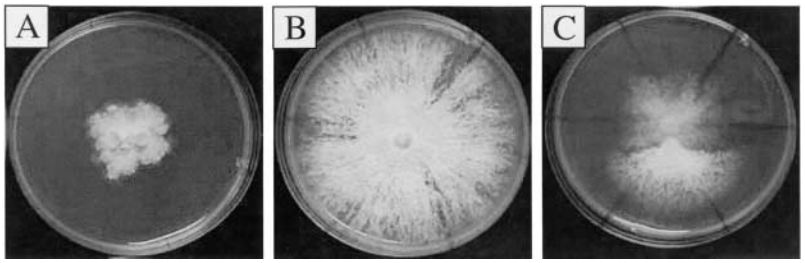

FIGURE 6.—(A–C). Distinct colony morphology of different matings of *S. commune* of equal age. (A) The growth rate and morphology of the progenitor dikaryon. (B) The increase of growth rate of the progenitor N1 paired with the dominant mutation on DIK-3 N2. (C) The dominant effect on growth rate and the "abnormal" colony morphology when DIK-3 N2 was paired with the DIK-1 N1 containing the mutation with deleterious effect.

In other dikaryons, clear evidence for recessive-lethal mutations was found. For example, all of the haploid nuclei recovered from DIK-1 at both 9 and 18 months were of the N2 type; the N1 type was not recovered among the 100 protoplast regenerates tested. Similarly, only the N1 nuclear type was recovered from DIK-10 at 18 months and from DIK-11 at 9 and 18 months. The N2 nuclear type was not present among the 98 and 100 protoplast regenerates tested from DIK-10 and DIK-11, respectively. The lethality was therefore not limited to a particular nuclear type.

The effect of the recessive-lethal mutations was also detected in germinating spores. The spore germination rates were analyzed for each of the 12 dikaryotic lines. The progenitor dikaryon had a spore germination rate of 96% (n = 152). The majority of dikaryotic lines had spore germination rates that were not significantly different from the progenitor (mean of 93%, SD 5.71). In contrast, the dikaryotic lines from which only one nuclear type was recovered among protoplast regenerates all exhibited significantly lower spore germination rates. The spore germination rates were DIK-1, 37% (n = 128) and 35% (n = 145) at 9 and 20 months, respectively; DIK-10, 48% (n = 149) at 9 months; and DIK-11, 46% (n = 156) and 48% (n = 138) at 9 and 18 months, respectively.

Pairings of the recovered haploid nuclear components of the dikaryons: To test if the haploid components of the dikaryotic lines adapt reciprocally to one another's presence, the nuclei were recovered from each of the six dikaryotic lines (DIK-1–DIK-6) and repaired with all haploids of compatible mating type, including: (a) evolved in the same dikaryon, (b) evolved in other dikaryons, (c) evolved as haploid monokaryons, and (d) progenitor haploid monokaryons. In no case did the pairing of the two nuclei that evolved together in a dikaryon result in a mycelium with the fastest growth rate. Among the 120 newly constructed dikaryons, the 12 fastest growth rates were all dikaryons with nuclei that evolved in separate cell dikaryons (see supplementary data at http://www.genetics.org/supplemental/). Although little variation was observed in the growth rates of the dikaryons resulting from the pairings of nuclear components from DIK-2, DIK-4, DIK-5, and DIK-6, considerable variation was observed among the dikaryons from pairings of the nuclear components of

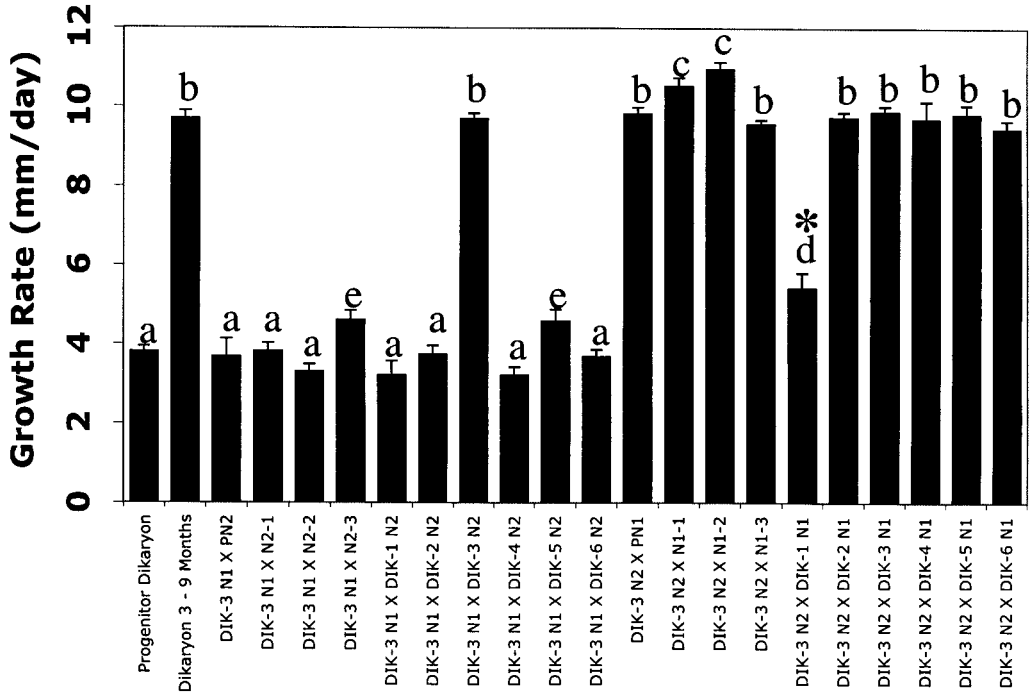

FIGURE 7.—Growth rates of the dikaryons formed from the compatible matings of nuclei recovered from the evolved dikaryon DIK-3. The dominant effect of the mutation for fast growth rate on DIK-3 N2 is clearly shown. The asterisk (*) indicates the abnormal colony morphology conferred by mating the DIK-3 N2 with the DIK-1 N1. Data shown are the mean ±SE of six measurements from six replicates; means denoted with the same letter are statistically similar ($P > 0.05$; one-way ANOVA with Tukey test).

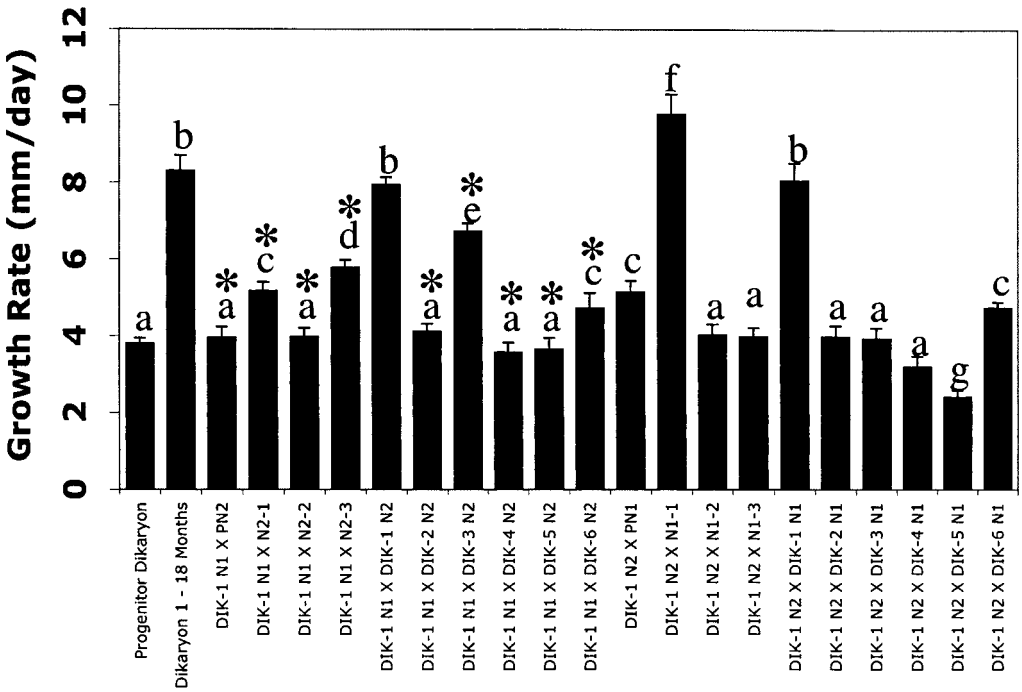

FIGURE 8.—Growth rates of the dikaryons formed from the compatible matings of neohaplonts recovered from the evolved dikaryon DIK-1. Asterisks (*) indicate the abnormal colony morphology conferred by the DIK-1 N1. Data shown are the mean ±SE of six measurements from six replicates; means denoted with the same letter are statistically similar ($P > 0.05$; one-way ANOVA with Tukey test).

DIK-1 and DIK-3, consistent with the mutations identified in these lines (see Figures 7 and 8). The growth rates of the nuclear components of each dikaryon were also determined as haploid monokaryotic cultures. None of the component nuclear types showed any increase or decrease in growth rates during this experiment when growing as monokaryons.

To test for cytoplasmic effects on growth rates, six replicates were made from each of the newly constructed dikaryons. Three replicates of each dikaryon were taken from opposite sides of the pairing, where the cytoplasm was that of the monokaryon but without identical nuclear genotypes. If the growth rates differed between the replicates taken from the same dikaryon, the phenotype could be attributed to a change in the cytoplasm (*i.e.*, mitochondrial). No cytoplasmic effects were observed in any of the pairings.

Although the evidence for reciprocal adaptation with respect to growth rates was not widespread, there was a strong interaction between the two component nuclei of DIK-1. Although the DIK-1 N1 nuclear type carried a recessive-lethal mutation, this nuclear type confers a sectoring, slow-growing phenotype in dikaryons from pairings with the compatible N2 nuclear types from all sources, except DIK-1 at 9 and 18 months (Figure 8). The restoration of a "normal" phenotype in the dikaryon from the pairing of DIK-1 N1 with DIK-1 N2 suggests that a compensatory mutation occurred in the N2 nuclear type of DIK-1 that overrides the deleterious effect of the N1 nucleus from DIK-1 (Figure 8). The observations above suggest that while the lethal effect from N1 is recessive, the deleterious effect on dikaryon morphology is dominant.

To test the interaction of mutations in DIK-1 at different time points, pairings were made from the component nuclei of DIK-1 before the deleterious mutation occurred (transfer 12), after the deleterious mutation occurred (transfer 14), at the time of the compensatory mutation (transfer 15), at the end of the experiment (transfer 39), and with the progenitor (see Figure 9). At transfer 12, both nuclei conferred a growth rate comparable to that of the progenitor dikaryon. At transfer

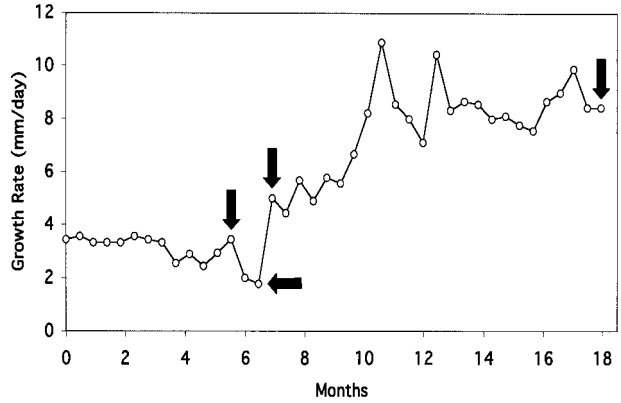

FIGURE 9.—Growth rate of DIK-1 over 18 months. At six months (transfer 13), mycelial growth rate decreased and colony morphology became abnormal due to a characterized deleterious mutation on N1. At ~7 months (transfer 15), mycelial growth rate and colony morphology were restored by a second, compensatory mutation on N2. The growth rate steadily increased during the experiment. Pairings with DIK-1 N1 taken after 6 months with nuclei from other lines confer the low growth rate and abnormal colony morphology. The arrows indicate time points of isolates used in crosses to identify mutations (transfers 12, 14, 15, and 39).

TABLE 2

Results of the multilocus genotyping: dikaryons with recombinant genotypes

Probe[a]	Neohaplont[b]	Allele[c]	Time point (mo)[d]
BG550561	DIK-4 N1	N2	18
	DIK-4 N2	N1	
BF942494	DIK-5 N1	N2	9 and 18
	DIK-5 N2	N1	
BG673937	DIK-6 N1	N2	18
	DIK-6 N2	N2	
BG713749	DIK-6 N1	N2	18
	DIK-6 N2	N1	
BF942494	DIK-6 N1	N2	18
	DIK-6 N2	N1	
BG447515	DIK-7 N1	N2	18
	DIK-7 N2	N2	
BF942494	DIK-8 N1	N2	18
	DIK-8 N2	N1	
BI135391	DIK-8 N1	N2	9 and 18
	DIK-8 N2	N1	
BG518461	DIK-12 N1	N2	18
	DIK-12 N2	N1	
BG550556	DIK-12 N1	N2	9 and 18
	DIK-12 N2	N1	

[a] Primer and probe conditions shown previously in Table 1.
[b] Isolated genotype is the haploid neohaplont recovered from the evolved dikaryon. The dikaryon of origin followed by the mating type of the isolate is given.
[c] Allele assigned from mating type of progenitor monokaryon that has specific hybridization to the corresponding oligonucleotide probe. N1 refers to the mating-type A1B1; N2 refers to the mating-type A2B2.
[d] Indicates time point of sampling where recombinant genotype was identified.

14, the pairings with the DIK-N1 nucleus had an "abnormal" phenotype only when paired with DIK-1 N2 from the progenitor, transfer 12, and transfer 14, but not from transfer 15 and transfer 39. These results are further evidence that a mutation occurred on the DIK-1 N2 nucleus with a compensatory effect on the mutations on the DIK-1 N1 nucleus.

To further investigate the nature of the compensatory effect, a series of crosses was made to dissect the genetics of the compensatory mutation. The nucleus with the compensatory effect DIK-1 N2 was mated with the progenitor N1 and the haploid progeny were screened with mating-type tester A1B1 until 97 were found with the mating-type A2B2. These 97 haploid progeny were separately mated with DIK-1 (9 months) to form dikaryons with the DIK-1 N1 nucleus conferring abnormal culture morphology. The resulting dikaryons were 38 abnormal:59 normal, a segregation ratio that is barely significantly different from 1:1 (chi-square test, $0.025 < P < 0.05$). Since the compensatory mutation may exert a potential bias in spore germination or may be linked to one of the mating-type genes, we do not conclude that the compensatory determinant is other than a single mutation at one locus.

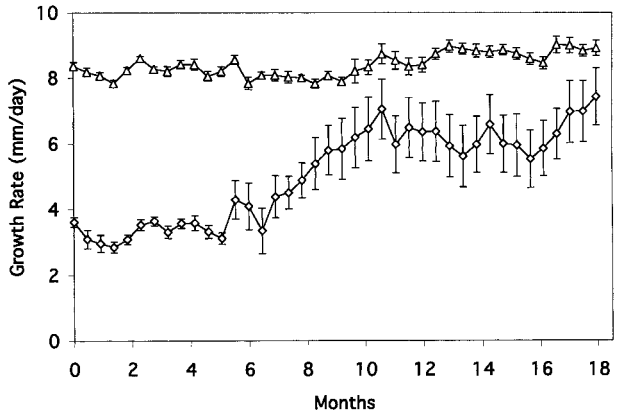

FIGURE 10.—Mean growth rates of all experimental lines; error bars indicate ±SE. The mean of the 12 haploid monokaryotic lines (△) showed minimal change during the course of the experiment while the mean of the 12 dikaryotic lines (◇) increased steadily from an initial growth rate of approximately half of the monokaryotic lines toward the mean growth rate at the end of the monokaryotic lines at the termination of the experiment.

Multilocus genotyping results: The multilocus genotypes of the experimental lines were unchanged in the monokaryotic lines and 10 cases of marker exchange between nuclei in the DIK-4, -5, -6, -7, -8, and -12 dikaryotic lines were found (Table 2). In the dikaryotic lines, 8 cases of reciprocal recombination were found and two regions were found to be homoallelic due to some unknown genetic mechanism (gene conversion, mitotic recombination). There were no instances of mating-type changes occurring in any of the dikaryotic lines.

DISCUSSION

In our experiments the response to selection for increased linear growth rate in *S. commune* was strongly determined by ploidy state of the mycelium: the dikaryons diverged to a much greater extent in growth rate than did the monokaryons during the 1.3×10^4 generations of this experiment. Several of the dikaryotic lines showed striking increases in growth rate, while the monokaryons, which had a faster growth rate at the start of the experiment, did not (see Figure 10). Furthermore, during the experiments the variance in growth rates increased to a much greater extent among the dikaryotic lines than among the monokaryotic lines. Also, in at least one dikaryotic line, a deleterious mutation in one nucleus was accompanied by a compensatory mutation in the other nucleus of the pair. In most cases, however, the two nuclei in the evolved dikaryons did not show any evidence for coadaptation. Finally, although the paired nuclei of each dikaryon remained distinct throughout the experiment, there were several examples of genetic exchange between nuclei. Taken together, all of the above results suggest that the dikaryotic phase of the life cycle of *S. commune* has a greater poten-

tial for expressing phenotypic change through mutation than does the haploid, monokaryotic stage.

The quantitative genetic basis of linear growth rate in haploid monokaryons and dikaryons of *S. commune* shows that the expression of genetic variation for growth rate is different in dikaryons and monokaryons. Artificial selection for growth rate in monokaryons (Simchen 1966; Connolly and Simchen 1968) and dikaryons (Simchen and Jinks 1964) indicates that growth rate has a polygenic basis, but that the phenotypic expression of the polygenes is different in each ploidy phase. Among dikaryons from a single population, additivity and dominance were the main sources of variance in growth rates (Simchen and Jinks 1964), while in monokaryons gene interactions (epistasis) were the main source of variance in growth rates (Simchen 1966). Further, dikaryons made from haploids taken from different geographic areas also showed epistatic interactions for growth rate (Simchen 1967). Additional evidence strongly suggests selection on polygenes for linear growth rate in *S. commune* in natural populations (Brasier 1970, 1987). The growth rates of dikaryons isolated from natural populations have a small range, yet crosses made from wild-collected dikaryons exhibited a far greater range of growth rates. These results strongly suggest that stabilizing selection acts upon dikaryotic growth rate. Taken together, the aforementioned work suggests that selection on growth rate elicits different responses in haploid monokaryons and dikaryons.

In contrast to studies examining natural variation in growth rates, our experiments began with a single dikaryotic genotype with no standing genetic variation among any of the replicate lines and measured fitness only in terms of linear growth rate. Although we relinquished the ability to evaluate total fitness under natural conditions in these experiments, we gained the ability to follow the responses to selection in real time and to identify their underlying genetic determinants in the context of a known history. All variability in our experiments was due only to mutation (in the broadest possible sense) that occurred within the time frame of the experiment in response to natural selection. Presumably, the potential for increase in linear growth rate through mutation is constrained to some inherent maximum at which one or more physiological processes, such as nutrient uptake or production of cell wall materials, become limiting. The responses of the monokaryons and dikaryons in our experiments were consistent with the existence of constraints, under which we interpret the monokaryons as remaining near their upper limit in growth rate and the dikaryons as approaching their limit during the experiments. This is best seen in Figure 10 where the mean growth rates of all monokaryotic lines are compared with those of the dikaryotic lines. The growth rates of the dikaryotic lines were initially approximately half that of the monokaryotic lines and through time approached the growth rates of the monokaryotic lines. This increase in mean growth rate of the dikaryons was not due solely to the dominant mutations in DIK-3 and DIK-8; other, unidentified mutations of smaller effect on growth rate are likely present.

In nature, the dikaryotic mycelium is the predominant stage of the basidiomycete life cycle. In *S. commune*, there are two states with respect to the morphology of dikaryotic mycelia: a slow-growing, asymmetrical colony (SA) type that occurs with even short exposure to daylight and a fast-growing symmetrical colony (FS) type that occurs in total darkness. For most of its life span, the dikaryotic mycelium of *S. commune* grows within woody substrates in darkness, presumably in the FS growth state. The light-induced SA growth state presumably occurs only when the dikaryotic mycelium grows out of the substrate and is exposed to light, at which time fruit bodies are produced. We suspect that, as the predominant, nutrient assimilating form, the FS state has been shaped more strongly than the SA state by stabilizing selection. The reason for this speculation is that, in our experiments in which light was present, the progenitor SA form in many cases changed to the FS form and at that point no further increases in growth rate occurred. Also, a minority of dikaryotic isolates from nature express the FS morphology constitutively in all environments. We interpret the monokaryotic growth form, which is the same in light and in darkness, as more similar to the dikaryotic FS than to the SA form.

The SA colony type appears to be controlled by an extracellular growth inhibitor (Klein *et al.* 1997), while the FS morphological types, including the mutant types observed here, apparently do not produce the inhibitor. The best explanation of the asymmetrical colony margin of dikaryons with the SA morphology is that there is a regulatory loop that cycles through (a) production of the inhibitor by the dikaryotic mycelium, (b) self-inhibition of the dikaryon due to the presence of the inhibitor, (c) reduced secretion of the inhibitor and lowering of extracellular concentrations due to diffusion outward, and (d) faster growth of the dikaryon and increased secretion. The dynamic and unstable nature of this regulatory loop best explains the lobate and asymmetric colony margin of the SA type. The dominant mutations in the DIK-3 and DIK-8 lines accompanied by the change from SA to FS types is best explained as failure to secrete the inhibitor. The alternate possibility of a failure to respond to the inhibitor is not consistent with pairing reactions between mutant FS dikaryons and monokaryons in which there is never a zone of growth inhibition separating the mycelia, as there invariably is between all SA dikaryotic types and monokaryons. The mutations identified in the DIK-3 and DIK-8 lines that effected the transition from the SA to the FS type are consistent with the hypothesis of Klein *et al.* (1997) that there is a genetic basis for specifying which of the two morphological states is expressed by the dikaryon.

The best candidate responsible for the change in colony growth rate and symmetry dikaryons is schizostatin, a diterpenoid compound isolated from *S. com-*

mune that is a potent and selective inhibitor of squalene synthase (Tanimoto *et al.* 1995, 1996). Squalene synthase is a major control point of isoprene and sterol biosynthesis in eukaryotes (Robinson *et al.* 1993) and an important enzyme in the creation of sterols required for fungal growth. Further investigation is needed to determine if schizostatin is indeed the growth inhibitor produced by asymmetrically growing dikaryons in the light.

In the dikaryotic mycelia, the two component nuclei coexist in the common cell environment, raising the possibility of coadapted gene combinations between haploid genomes. Theoretical predictions by Kimura and Crow (1965) indicate that asexual organisms would accumulate coadapted complexes through complementary gene action during selection. Our experiments provided controlled environments and known histories to test whether or not the haploid components of the dikaryon adapted reciprocally to one another's presence over time. The dikaryotic ploidy state allowed for this prediction to be tested in a way that is unavailable to diploid organisms. Our protocol allowed both haploid components of a dikaryotic line to be recovered intact, without meiosis, and then paired with nuclei of different histories to create new dikaryons.

In one case, there was strong evidence for one coadapted pair of genes in different haploid genomes. In the DIK-1 line, two mutations, first a deleterious change in one nuclear component followed by a compensatory change in the other nuclear component, occurred sequentially, as predicted by Kimura and Crow (1965). Whether or not such coadaptation occurs between the haploid components of dikaryons in nature is not yet known, but could be tested in long-lived mycelia such as those of fairy rings. The alternate possibility to coadaptation is that the nuclear components of dikaryons can be readily shuffled in dikaryon-monokaryon matings to produce new dikaryons capable of persisting (Buller 1931, 1941). The conditions for flexibility in nuclear exchange and reassociation were found in the high fitness of the component nuclear types of the dikaryons other than DIK-1 in new combinations. Not only was fitness not impaired in most of the new combinations of nuclear types, but also, surprisingly, these new combinations often had the fastest growth rates. This could be a result of a heterozygote advantage from the allelic differences. This is consistent with observations that in pairings between dikaryons and monokaryons in which all nuclei have compatible mating types, there is often nuclear selection in which nuclei that are the least similar genetically tend to pair to form new dikaryons (Raper 1966).

The persistence of the coadapted genes in the dikaryotic lines would be affected by somatic recombination and meiotic recombination. If genes were shuffled between the component nuclei, coadapted gene complexes could be either broken up or made to be even more genetically dependent. The impact of coadaptation in dikaryons would then be expected to be strongly influenced by the length of time of asexual propagation and the breeding structure of the population. If coadapted gene complexes in dikaryons did occur in nature at high frequency, dikaryons formed through mating of unrelated haploid progeny would be expected to be maladapted.

To complement our measurements of the response to selection for increased growth rates and our observation of coadaptation between the nuclei in a dikaryon, we also asked whether or not genetic exchange occurred between the two nuclei in the dikaryons during the time of our experiments. Adaptive change depends on genetic variability and the phenotypic selection that comes from this genetic variability. Mechanisms like somatic recombination could contribute to genotypic (as opposed to allelic) variability and may increase the rate of adaptation. For example, environmental stresses and pathogen infection have been shown to increase somatic recombination in plants (Lucht *et al.* 2002; Kovalchuk *et al.* 2003) and somatic recombination has been described in several fungal species, including *S. commune* (reviewed by Ellingboe 1965). The impact of the episodic recombination events, including both reciprocal exchange and loss of heteroallelism, observed in our experimental lines on fitness is not yet known. The impact could be positive or negative, depending on whether the new gene combinations were beneficial and/or if beneficial gene combinations were disrupted. Additional, undetected recombination events may well have occurred in the 12 dikaryotic lines and could be a factor related to the increase in growth rates in the dikaryotic lines.

Our experiments show that the dikaryotic basidiomycete mycelium can respond to selection for increase in growth rate, that there is evidence of coadaptation between nuclei in a dikaryotic mycelium, and that the two nuclei in a dikaryotic mycelium do undergo episodic somatic recombination during vegetative growth. Of the two vegetative forms of this fungus, haploid monokaryotic and dikaryotic, the latter was more responsive to selection for increased growth. Mutation, coadaptation, and somatic recombination may all contribute to the phenotypic plasticity of the dikaryon. These processes may allow the dikaryon to function as a kind of capacitor for evolutionary change through the masking and then unmasking of allelic and genotypic variation. The genetic plasticity and the ability to undergo nuclear reassortment without meiosis have been hypothesized to permit dikaryons to have a critical selective advantage over diploidy (Raper and Flexer 1970). We are currently conducting experiments to test if the adaptive potential of the dikaryotic state is greater than that of the corresponding diploid state. The hypothesis is that dikaryons and diploids differ with respect to their patterns of genetic change over time, with accompanying

differences in the patterns of gene regulation and phenotypic plasticity. Our next goal is to compare the evolutionary potentials of dikaryons and diploids on a level playing field with isogenic strains.

We thank Vera Dounaevskaia and Caroline Sirjusingh for technical assistance. This work was supported by a research grant from the Natural Sciences and Engineering Research Council of Canada to J.B.A.

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Communicating editor: P. J. Pukkila