Pseudohomothallism and Evolution of the Mating-Type Chromosome in *Neurospora tetrasperma*

Sandra T. Merino,* Mary Anne Nelson,* David J. Jacobson,[†,1] and Donald O. Natvig*

**Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131 and [†]Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824*

Manuscript received October 31, 1995
Accepted for publication March 8, 1996

ABSTRACT

Ascospores of *Neurospora tetrasperma* normally contain nuclei of both mating-type idiomorphs (*a* and *A*), resulting in self-fertile heterokaryons (a type of sexual reproduction termed pseudohomothallism). Occasional homokaryotic self-sterile strains (either *a* or *A*) behave as heterothallics and, in principle, provide *N. tetrasperma* with a means for facultative outcrossing. This study was conceived as an investigation of the population biology of *N. tetrasperma* to assess levels of intrastrain heterokaryosis (heterozygosity). The unexpected result was that the mating-type chromosome and autosomes exhibited very different patterns of evolution, apparently because of suppressed recombination between mating-type chromosomes. Analysis of sequences on the mating-type chromosomes of wild-collected self-fertile strains revealed high levels of genetic variability between sibling *A* and *a* nuclei. In contrast, sequences on autosomes of sibling *A* and *a* nuclei exhibited nearly complete homogeneity. Conservation of distinct haplotype combinations on *A* and *a* mating-type chromosomes in strains from diverse locations further suggested an absence of recombination over substantial periods of evolutionary time. The suppression of recombination on the *N. tetrasperma* mating-type chromosome, expected to ensure a high frequency of self fertility, presents an interesting parallel with, and possible model for studying aspects of, the evolution of mammalian sex chromosomes.

IN 1927, SHEAR and DODGE described a new ascomycetous fungal genus, Neurospora, in an article that included morphological species descriptions as well as detailed genetic analyses of sexual cycles (SHEAR and DODGE 1927). One of the species, *Neurospora crassa,* later became an important organism for studies of genetics and biochemistry. The *N. crassa* sexual cycle is typical of that of the truly heterothallic (self-sterile) species of the genus (reviewed by RAJU 1980, 1992b). Sexual reproduction results in a diploid nucleus that undergoes meiosis and a subsequent mitosis to form eight haploid ascospores, which are contained within a specialized structure called an ascus. The contents of an *N. crassa* ascus therefore represent a meiotic tetrad, elaborated to four pairs of ascospores by the postmeiotic mitosis. An ascospore germinates to produce a vegetative mycelium that is haploid and homokaryotic (possessing genetically identical nuclei). Mating in *N. crassa* is controlled by a single multi-gene locus on the largest chromosome, representing linkage group I, which we refer to here as the mating-type chromosome. Successful matings occur only between strains with different sequences at the mating-type locus. The two alternative sequences at this locus, *A* and *a,* historically were referred to as alleles. However, they are now termed idiomorphs, because the encoded structural genes do not share detectable sequence homology (GLASS *et al.* 1990; METZENBERG and GLASS 1990; STABEN and YANOFSKY 1990). During meiosis, the *A* and *a* idiomorphs segregate, with the result that each ascus possesses four *A* and four *a* ascospores.

The mating-type chromosomes of the heterothallic species in the genus Neurospora differ substantially in the regions of the *a* and *A* mating-type idiomorphs (GLASS *et al.* 1988). In their possession of nonhomologous regions that control critical aspects of sexual reproduction, in addition to homologous regions, the mating-type chromosomes resemble the sex chromosomes of heterogametic organisms such as humans. In the interest of clarity and expediency, we refer in this article to nonmating-type chromosomes of Neurospora as autosomes.

Among the species described by SHEAR and DODGE, *N. crassa* and *N. sitophila* were eight-spored heterothallics with the life cycle described above. In contrast, another species, *N. tetrasperma,* produced asci with four spores that were larger than those of the eight-spored species. These ascospores germinated to produce self-fertile (homothallic) mycelia. SHEAR and DODGE made two important observations that provided the first strong evidence that the mycelium of *N. tetrasperma* is a heterokaryon containing separate nuclei of the two mating types (DODGE 1927; SHEAR and DODGE 1927). First,

Corresponding author: Donald O. Natvig, Department of Biology, University of New Mexico, Albuquerque, NM 87131.
E-mail: dnatvig@unm.edu

[1] *Present address:* Department of Biological Sciences, Stanford University, Stanford, CA 94305.

they demonstrated that occasional small ascospores germinated to produce strains that were self-sterile and that in fact were heterothallic, producing successful matings only when crossed with similarly derived mycelia of opposite mating type. These homokaryotic ascospores typically appear in asci that possess three heterokaryotic ascospores and two smaller homokaryotic ascospores, instead of the usual four. Second, SHEAR and DODGE obtained evidence for the evolutionary conservation of mating-type genes among species in the genus by demonstrating that self-sterile *N. tetrasperma* mycelia could initiate mating with either *N. crassa* or *N. sitophila,* but only when the proper mating-type combinations were employed. The conservation of the *A* and *a* idiomorphs in *N. tetrasperma* was later proven by introgression into *N. crassa* (METZENBERG and AHLGREN 1973).

The formation of self-fertile (*A* + *a*) ascospores in *N. tetrasperma* is now understood to derive from programmed meiotic spindle alignment, nuclear movement and ascospore delimitation, with the result that each ascospore receives one nucleus of each mating type during ascus development. Each ascospore receives *a* and *A* nuclei produced from a single diploid nucleus. The fidelity of this process is dependent on the segregation of *a* and *A* idiomorphs at the first division of meiosis (Figure 1A). Although ascus development differs in important respects between *N. tetrasperma* and the eight-spored species, chromosome structures of *N. tetrasperma* are virtually indistinguishable from those of *N. crassa,* and gene order appears to be conserved (HOWE and HAYSMAN 1966; PERKINS 1985; discussed below).

Apparent homothallism (self fertility) of the general type exhibited by *N. tetrasperma* is referred to as pseudohomothallism or secondary homothallism. It has arisen independently in diverse fungi, including *Podospora anserina* and *Gelasinospora tetrasperma,* two organisms placed in the same ascomycetous family as Neurospora (Sordariaceae), as well as the common commercial mushroom *Agaricus brunnescens* (Langton and Elliott 1980). However, the genetic and cellular mechanisms of pseudohomothallism differ substantially.

In *N. tetrasperma,* as many as 10% of the ascospores and 20% of the conidia receive only one nuclear type and are therefore self-sterile. Thus, there would appear to be excellent potential for facultative outcrossing in nature (Raju 1992a; Raju and Perkins 1994). The present study was initiated to assess the level of intrastrain genetic variability[2] in wild-collected strains of *N. tetrasperma* toward the goal of estimating the degree of outcrossing. Quite separate from the question of outcrossing potential, our results revealed that *N. tetrasperma* possesses a pattern of chromosome evolution that is most readily explained by suppressed recombination on the mating-type chromosome. Recombination on *N. tetrasperma* autosomes appears to be normal relative to other members of the genus. This pattern of chromosome evolution presents both an interesting case of adaptation to accommodate a particular life history and an example of chromosome evolution that in several respects parallels that of mammalian sex chromosomes.

[2] We have avoided the terms *heterozygous* and *homozygous* in this context. Most cells of *N. tetrasperma* are heterokaryotic, not diploid, and for that reason, these terms are considered by some geneticists to be inappropriate for stages in the life cycle other than the fusion nucleus of the ascus.

MATERIALS AND METHODS

Strains: We employed 11 heterokaryotic (parental) *N. tetrasperma* strains representing diverse locations. Each heterokaryon was separated into a pair of *A* and *a* homokaryotic sibling strains. One pair (85*a*, 85*A*) consisted of designated species-tester strains (PERKINS, TURNER and BARRY 1976) that were derived from a common laboratory parental strain of unknown geographic origin. The other 10 pairs were chosen from the collection of DAVID PERKINS, Stanford University (PERKINS 1976; PERKINS and TURNER 1988). Parental strains were chosen to represent a range of geographic separation from same-site to different continents. A more complete description of the strains and their origins is given by JACOBSON (1995).

Genetic analysis: Genetic variability at specific loci was determined by assaying restriction fragment length polymorphisms (RFLPs) in *N. tetrasperma* sequences homologous to cloned sequences from *N. crassa.* All but one of these sequences were derived from the genomic cosmid library of VOLLMER and YANOFSKY (1986). The cosmid sequences included four that were mapped in *N. crassa* to the mating-type chromosome and three others that mapped to autosomes (Figure 2). An eighth sequence was obtained as a lambda phage clone (λL-1A) carrying a portion of the *N. crassa qa* gene cluster (GILES *et al.* 1985), generously provided by ROBERT GEEVER, University of Nevada. This sequence was chosen specifically to examine polymorphism in a sequence that maps close to the centromere of an autosome (Figure 2).

Genomic *N. tetrasperma* DNAs were purified using the method of STEVENS and METZENBERG (1982). Cosmid DNAs were labeled with digoxigenin-tagged dUTP, and blot hybridization and detection were performed using the Lumiphos system of Boehringer-Mannheim.

Tree-building methods: RFLP data were subjected to tree-building analyses using several different parsimony (bootstrap and nonbootstrap) and distance procedures (Fitch, Kitsch and UPGMA) available in PAUP version 3.1 (SWOFFORD 1993; partial results presented here) and PHYLIP version 3.5 (FELSENSTEIN 1993; results not shown), respectively. For these analyses, individual restriction fragments were scored as either present or absent in each of the 22 homokaryotic strains (APPENDIX). Fragments were assumed to be identical if their mobilities were indistinguishable. Parsimony analyses with PAUP employed matrices of presence/absence scores. Distance values for nonparsimony analyses were obtained with the same matrices using the PAUP "Show Distance Matrix" option.

The scoring of restriction fragments as individual characters in principle is less desirable than scoring based on the presence or absence of individual restriction sites, because in the former case individual characters are frequently not independent (SWOFFORD and OLSEN 1990). The method of scoring employed here was required by the large data set. The presentation of trees is intended primarily to dramatize the different patterns of evolution observed for the mating-type chromosomes and autosomes, and only secondarily to suggest possible phylogenetic relationships.

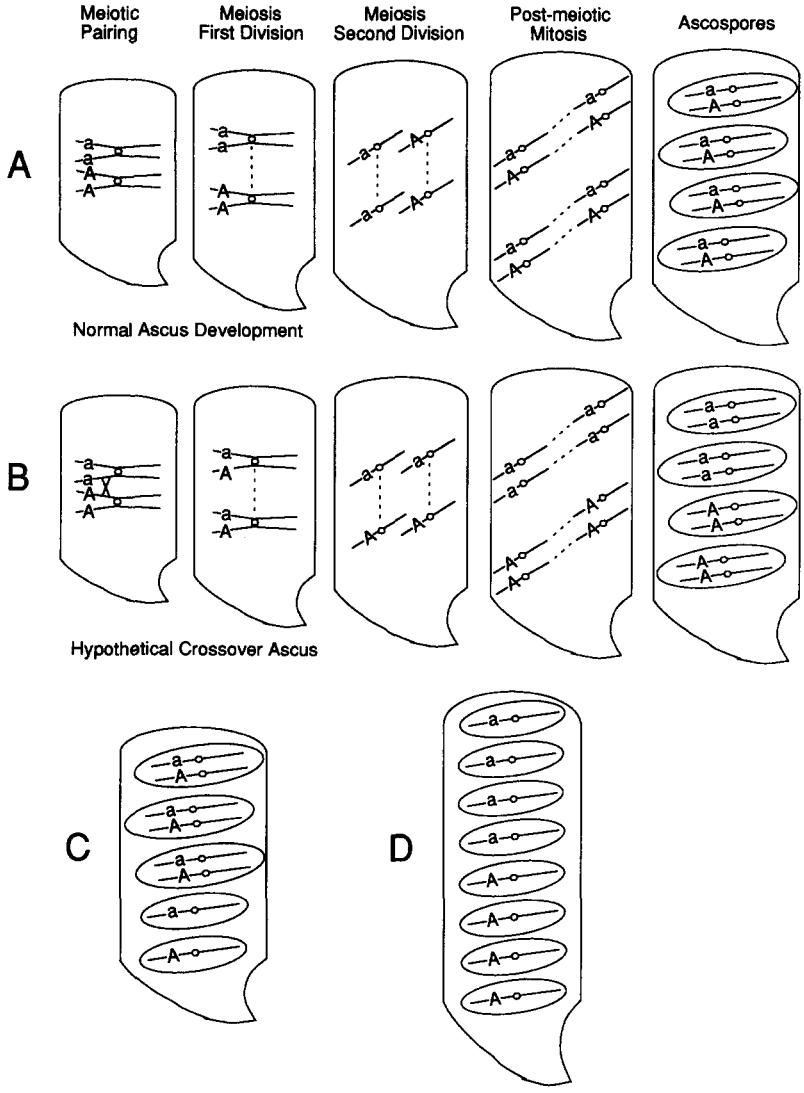

FIGURE 1.—Ascus development and pseudohomothallism in *N. tetrasperma*. (A) Programmed spindle alignment and nuclear positioning normally lead to delimitation of four heterokaryotic ascospores (nuclear envelopes are not shown). We note that mature ascospores actually possess several nuclei of each mating type as a result of mitotic divisions that occur during spore maturation. (B) The mechanism by which a crossover between the mating-type locus and the centromere (shown in youngest ascus) would result in the formation of single mating-type ascospores. In the same manner, repeated crossing over on autosomes can lead to loss of variability at other loci. The genetic composition of ascospores in this hypothetical example reflects the polarity of chromatid separation at the second meiotic division. This ascospore arrangement would be expected in one-half of the crossover asci. There is an equal probability of obtaining a normal (*a* + *A*) arrangement. (C) An occasional *N. tetrasperma* ascus possesses three large heterokaryotic ascospores and two small homokaryotic ascospores. (D) A typical ascus of an eight-spored, truly heterothallic species such as *N. crassa* is shown for comparison.

RESULTS AND DISCUSSION

Different patterns of evolution for different chromosomes: Our results present an intriguing and somewhat complex picture of chromosome evolution among different *N. tetrasperma* strains. Most striking is the observation that hybridization with probes from the mating-type chromosome produced results very different from those obtained with autosomal probes. In nearly every instance, RFLP differences were observed in sibling *A* and *a* nuclei when we hybridized with any of four mating-type chromosome probes (Figure 3A and APPENDIX, Figure A1 and Table A1), demonstrating a high degree of intrastrain polymorphism for linkage group I sequences. Trees produced based on RFLPs among these mating-type chromosome sequences revealed clustering primarily influenced by mating type and only secondarily influenced by geography (Figure 4A). In contrast, sequences from three autosomes exhibited nearly complete intrastrain homogeneity (Figure 3B; APPENDIX). Trees produced based on autosomal RFLPs revealed close affinities between sibling *a* and *A* strains, and the observed clustering reflected the geographical origins of the strains, with the caveat that substantial genetic differences were observed among subgroups from Louisiana (Figure 4B). The general features of the trees presented, including all features relevant to the conclusions presented in this article, were the same with all methods of analysis. These included bootstrap and nonbootstrap parsimony methods with PAUP, and Fitch, Kitsch and UPGMA methods with PHYLIP (results summarized in Figure 4 legend). The results were so consistent that our initial DNA preparation from Hawaiian strain P556*a*, which gave an apparent anomalous result in preliminary analyses, was first suspected, and then confirmed, to be derived from a mixed culture contaminated with a strain from Louisiana (Figure 3). The very different modes of evolution exhibited by mating-type chromosomes and autosomes are also revealed by the results of an analysis in which we assigned a haplotype designation to each unique variant observed with cosmid probes (Table 1; APPENDIX). The combined results provide dramatic evidence that correct interpretations of the course of evolution, drawn from comparative-genetic information, are subject to pitfalls when based on data from single loci or on invalid assumptions

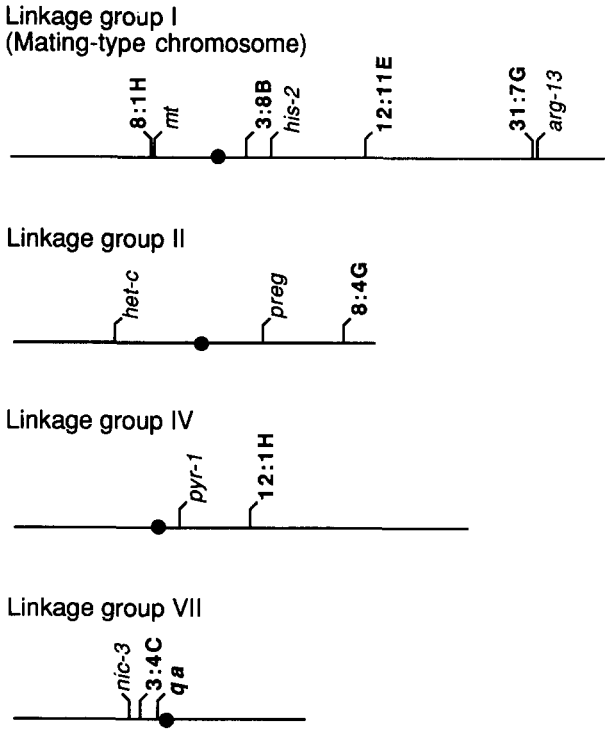

FIGURE 2.—Map locations of cloned sequences used as hybridization probes (shown in bold) and several reference loci (presented with reference to *N. crassa* linkage groups). Noncosmid gene locations are from PERKINS *et al.* (PERKINS *et al.* 1982; PERKINS 1992), except for the location of the *qa* gene cluster, which is from CENTOLA and CARBON (1994). Locations for cosmid sequences were determined by multicent mapping (METZENBERG *et al.* 1984; METZENBERG and GROTELUESCHEN 1993). The map is drawn approximately to scale. ●, the positions of centromeres.

(expressed or implied) regarding life history or genome structure.

Suppressed recombination on the mating-type chromosome: There appears to be a near or total absence of recombination among the loci examined on the mating-type chromosome. Strong evidence for this is observed as conserved cosmid-defined haplotype combinations on *A* and *a* chromosomes from diverse parental strains (Table 1). The conservation of haplotype combinations, along with mating type, is most readily apparent among four parental strains, P2356 and P2361 from New Zealand, and P586 and P583 from Hawaii. Despite the fact that the regions examined span a substantial distance on the *N. crassa* mating-type chromosome (Figure 2), the results suggest no recombination among them since the evolutionary divergence of these strains. Similarly, we found no evidence for recombination among these loci in the other *N. tetrasperma* strains examined. This is most evident in the results presented in Figures 3A and 4A; it is less clear in Table 1, wherein close affinities among certain haplotypes are obscured by the summary presentation.

Our results complement those of crossing experiments reported by HOWE (1963) and HOWE and HAYSMAN (1966), who observed reduced recombination among several linkage-group I genes in *N. tetrasperma* relative to that observed in *N. crassa*. For example, among 154 progeny derived from homokaryotic ascospores, no recombination was observed between an albino locus and the mating-type locus. Similarly, no recombination was observed between the mating-type locus and the gene for cycloheximide resistance in 42 progeny (see Table 3 in HOWE and HAYSMAN 1966). The *N. crassa* loci for cycloheximide resistance and albino are on the opposite chromosomal arm from the mating-type locus, and they exhibit recombination frequencies ≥40% with the mating-type locus (PERKINS *et al.* 1982; D. PERKINS, personal communication). HOWE and HAYSMAN suggested that relative to *N. crassa*, *N. tetrasperma* might possess rearrangements on the mating-type chromosome. Given the distribution of loci examined in our study and this previous study, it now appears that the observed results reflect a more general suppression of recombination on the *N. tetrasperma* mating-type chromosome, rather than differences in gene arrangement involving large segments of the chromosome. Our results do not preclude the possibility that small local rearrangements contribute to suppression of recombination. The results of HOWE and HAYSMAN and those reported here (see below) argue for normal levels of recombination on autosomes.

Suppression of recombination on the mating-type chromosome of *N. tetrasperma* should be adaptive toward the goal of preserving self-fertility. Ordered nuclear movements during meiosis in this species, resulting in spindle overlap during the second meiotic division, typically produce heterokaryotic self-fertile ascospores, wherein *a* and *A* nuclei have been derived from a single parental diploid nucleus (Figure 1A; RAJU 1992a). This pathway is dependent on first division segregation of mating-type idiomorphs. A crossover between the mating-type locus and the centromere would permit production of ascospores with either two *a* or two *A* nuclei with a concomitant loss of self fertility (Figure 1B). Assuming that gene locations are conserved among members of the genus Neurospora, such recombination events are predicted to be relatively common, given the recombination frequency (>10%) between the mating-type locus and centromere in *N. crassa* (PERKINS *et al.* 1982). We note that recombination on autosomes will in like manner produce ascospores homokaryotic for loci on those chromosomes but without loss of self-fertility.

To the extent that suppressed recombination on the mating-type chromosome of *N. tetrasperma* reflects selection against ascospores that are homokaryotic for mating type, this mechanism of reinforcing pseudohomothallism is opposite that observed for other pseudohomothallic ascomycetes. *P. anserina* and *G. tetrasperma* appear to produce ascospores heterokaryotic for mating type as a result of an obligatory crossover that ensures second division segregation of opposite mating-type loci (reviewed by RAJU and PERKINS 1994). Taken

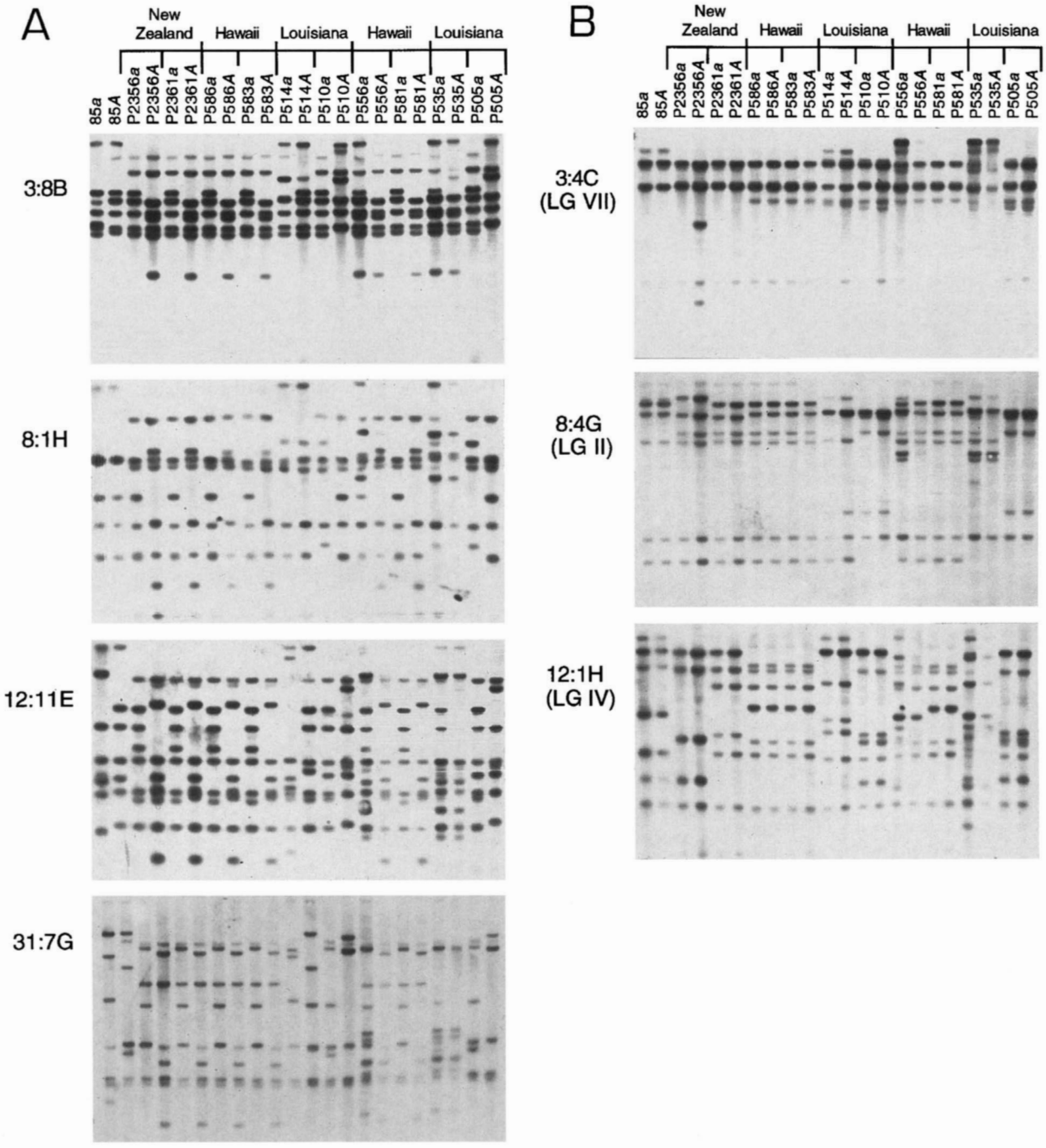

FIGURE 3.—RFLP patterns revealed by hybridization with digoxigenin-labeled cosmid sequences. Strains are arranged such that (starting from the left) adjacent *a* and *A* strains are siblings derived from single wild-collected parental strains. The locations of origin are shown (the origin of parental strain 85 is unknown). All genomic DNAs were digested with *Hin*dIII. Cosmid probes are indicated in the left margin. (A) Results obtained with mating-type chromosome probes. Note frequent differences between *a* and *A* sibling strains, and frequent similarities between nonsibling strains of the same mating type. (B) Results obtained with autosomal probes (LG, linkage group). Note that sibling *a* and *A* strains typically have identical patterns. The lanes representing Hawaiian strain P556*a* possess extra bands that were discovered to result from contaminating DNA from strain P535 from Louisiana (discussed in text). Contaminating bands, identified by replicate experiments with DNA preparations from single-conidium isolates, were not scored in tree-building analyses.

together, however, the modified recombination systems of *N. tetrasperma, P. anserina* and *G. tetrasperma* appear to reflect strong natural selection for reduced occurrence of ascospores homokaryotic for mating type.

Reduced recombination has been proposed as a mechanism for maintaining pseudohomothallism in *Agaricus bisporus* (SUMMERBELL *et al.* 1989; KERRIGAN *et al.* 1993). It appears that *N. tetrasperma* differs from *A. bisporus* in possessing a greater level of suppression and in having suppression restricted to the mating-type chromosome.

To date we have little information concerning the

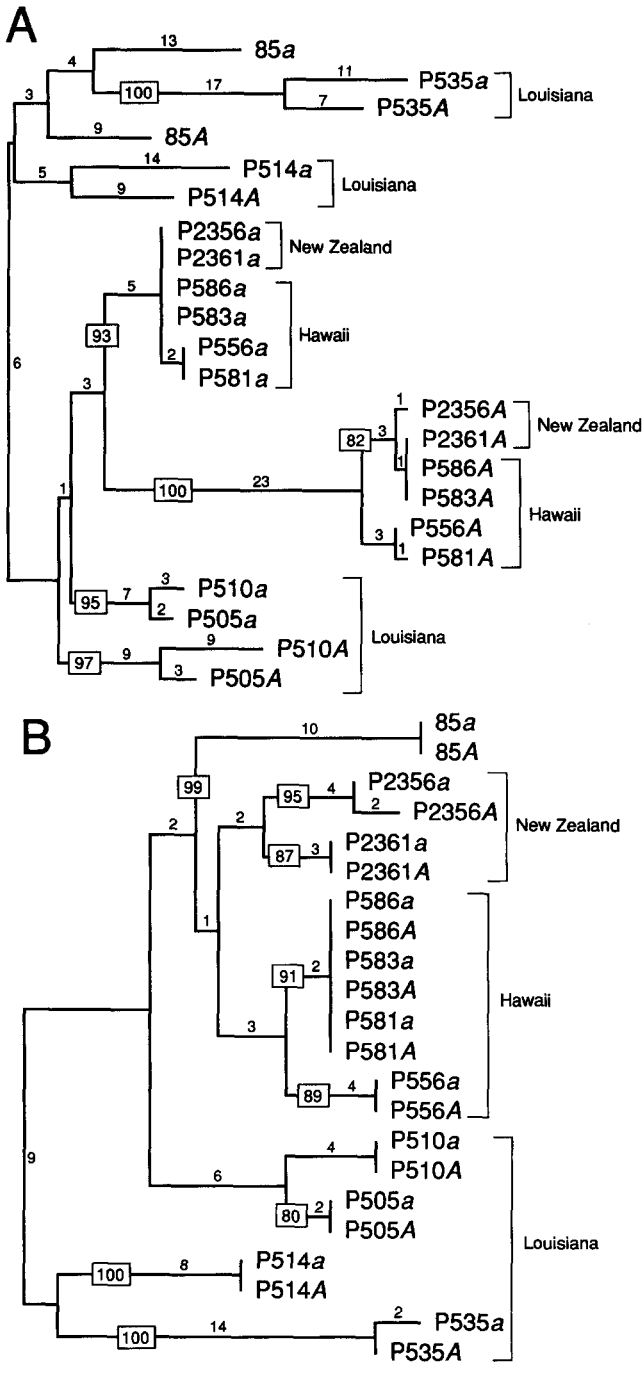

FIGURE 4.—Parsimony trees constructed from RFLP data. (A) Tree constructed based on the mating-type chromosome sequences. Note clustering based primarily on mating type. (B) Tree constructed based on autosomal sequences. Note the strong similarity typically observed between sibling *a* and *A* strains, as well as clustering based on geographical origins. The trees presented are 50%-majority-rule consensus trees obtained from bootstrap (100 data sets) parsimony (heuristic) analysis using PAUP. Boxed numbers represent the percentage of bootstrap trees that support the grouping of strains to the right of the particular internode. Only values ≥80% are shown. Other numbers represent the number of steps (or differences) along given branches as deduced by parsimony analysis. The trees were rooted using the PAUP "midpoint" option. Nonbootstrap parsimony analysis (heuristic) of the mating-type chromosome data produced 12 equally parsimonious trees (length = 173, consistency index = 0.532), one of which was identical to the bootstrap tree presented here. All trees derived from the mating-type chromosome, including distance trees, possessed two clusters of New Zealand and Hawaiian strains, one comprised of all *a* and another comprised of all *A* strains from these locations. All of these trees also possessed three relatively long branches representing three pairs of strains (P535*a*, P535*A*; P510*a*, P505*a*; and P510*A*, P505*A*). Sibling strains P514*A* and P514*a* shared a node (were closest neighbors) in Fitch and all parsimony trees, but not in Kitsch and UPGMA trees. Sibling strains 85*A* and 85*a* shared a node in Fitch, UPGMA and six of 12 maximum parsimony trees. Nonbootstrap parsimony analysis of the autosomal data produced five equally parsimonious trees (length = 74, consistency index = 0.595), none of which was identical to the bootstrap tree presented here (length = 76, consistency index = 0.579). The trees derived from autosomal data all exhibited separate clusters of strains from Hawaii and New Zealand, as is shown here in B. Not surprisingly, all *A* and *a* siblings were nearest neighbors in all trees. The general branching pattern observed for strains from Louisiana was the same in all except the UPGMA tree, which placed the pair of P514 siblings closer to the P505 and P510 strains than to the P535 strains. The data matrices employed (representing band scorings by strain) and a list of restriction fragments by size are presented in the APPENDIX.

mechanism by which recombination is suppressed on the mating-type chromosome. We examined the possibility that DNA methylation is responsible by employing methylation-sensitive and -insensitive restriction endonucleases that recognize the same base pair sequence (GATC). We found no evidence that the sequence examined (that corresponding to cosmid probe 8:1H of the mating-type chromosome) is methylated in either *a* or *A* strains (Figure 5).

Although the genetic data reported by HOWE and HAYSMAN (1966) revealed reduced recombination relative to *N. crassa* on the mating-type chromosome, a small number of recombinational events were detected between mating-type-chromosome genes. This observation that recombination is greatly reduced, but not abolished, may help explain the complex nature of the tree we obtained using sequences from mating-type chromosomes (Figure 4A). For example, siblings P535*a* and P535*A* grouped together, as did P514*a* and P514*A*, even though the siblings exhibited numerous differences with respect to each other (as indicated by branch lengths). This relationship may reflect interplay over evolutionary time between the competing forces of recombination (working to homogenize sibling mating-type chromosomes) and mutation (working to create divergence).

Implications for *N. tetrasperma* population biology: Do our results permit assessment of outcrossing in *N. tetrasperma*? There are two reasonable interpretations of our data. The observed homogenization of autosomal sequences could result either from selfing with crossing over (on the autosomes) or from matings among closely related individuals at the population level as a result of facultative heterothallism. It is instructive to consider the potential genetic consequences of the reproductive system of this organism. Paradoxically, *in the absence of crossing over,* pseudohomothallism in *N. tetrasperma* can

TABLE 1

***N. tetrasperma* haplotypes revealed by cosmid probes**

Cosmid	Strain and location[a]																			
	85		New Zealand				Hawaii				Louisiana				Hawaii				Louisiana			
			P2356		P2361		P586		P583		P514		P510		P556		P581		P535		P505	
	a	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*	*a*	*A*
Mating-type chromosome sequences																						
12:11E	B1	B2	B3	B4[b]	B3	B4	B3	B4	B3	B4	B5	B6	B7	B8	B3	B9	B3	B4	B10	B11	B7	B12
3:8B	C1	C2	C3	C4	C3	C4	C3	C4	C3	C4	C5	C6	C3	C7	C8	C9	C8	C9	C10	C11	C12	C13
8:1H	D1	D1	D2	D3	D2	D3	D2	D3	D2	D3	D4	D5	D6	D7	D2	D3	D2	D3	D8	D9	D10	D11
31:7G	E1	E2	E3	E4	E3	E4	E3	E4	E3	E4	E5	E6	E7	E8	E3	E4	E3	E4	E9	E10	E11	E12
Autosomal sequences																						
8:4G	F1		F2	F2	F3	F3	F4	F4	F4	F4	F5	F5	F6	F6	F7	F7	F4	F4	F8	F8	F9	F9
12:1H	G1	G1	G2	G2	G3	G3	G4	G4	G4	G4	G5	G5	G6	G6	G7	G7	G4	G4	G8	G8	G9	G9
3:4C	H1	H1	H2	H2[c]	H2	H2	H3	H3	H3	H3	H4	H4	H5	H5	H3	H3	H3	H3	H6	H6	H5	H5

Haplotypes were designated based on RFLP patterns generated from the blot hybridizations presented in Figure 3. Each unique variant was given a specific designation.

[a] The origin of strain 85 is unknown.

[b] The pattern exhibited was clearly a minor variant of that given the same designation in other strains.

[c] The hybridization profile of this strain revealed an additional band that might indicate a duplication of one portion of the sequence represented by the cosmid. The pattern was otherwise identical to that of the sibling strain, P2356*a*.

FIGURE 5.—Examination of DNA methylation associated with a region on the mating-type chromosome. DNAs from sibling *a* and *A* strains were digested with the methylation-sensitive enzyme *Mbo*I and the methylation-insensitive enzyme *Sau*3AI. Blot hybridization was performed with labeled DNA from the cosmid 8:1H (which maps near the mating-type locus). The strong similarities between *Sau*3AI- and *Mbo*I-digested DNAs from single strains suggest an absence of methylation at the sites examined.

serve to preserve existing genetic variability at any locus on any chromosome, while at the same time it ensures selfing. The nuclear behavior associated with ascus development in *N. tetrasperma* normally results in heterokaryotic ($A + a$) ascospores, wherein homologous chromosomes of the *A* and *a* nuclei in a given ascospore do not represent sister chromatids (Figure 1A; RAJU 1992a). As a consequence, independent assortment will permit a given autosomal gene to change its mating-type chromosome affiliation from generation to generation, but independent assortment alone will not act to produce paired *a* and *A* nuclei with autosomal loci derived from sister chromatids. Consider, for example, an autosomal gene *X*, with alleles *X* and *x*, together with the mating-type chromosome idiomorphs *A* and *a*. Let $[AX + ax]$ represent a single heterokaryotic ascospore in which one nucleus possesses *A* and *X* while the other possesses *a* and *x*. In the absence of crossing over, pseudohomothallic reproduction could alternately produce $[AX + ax]$ and $[aX + Ax]$ ascospore combinations, in either case preserving variability at *X*, whereas $[AX + aX]$ and $[Ax + ax]$ combinations will not occur.

This mechanism for preserving genetic variability would not apply to facultative heterothallic matings between the occasional mating-type homokaryons that arise in a given population. Therefore, whereas pseudohomothallism can help to preserve *intra*strain variability, heterothallic matings between closely related homokaryons potentially would produce a quite different result, namely, extensive genetic homogeneity in recon-

stituted heterokaryons. This homogeneity would be restricted to autosomes, since successful matings occur only when strains differ at the mating-type locus. A given strain would remain heterokaryotic with respect to the mating-type idiomorph and, in the absence of crossing over, with respect to other loci on the mating-type chromosome.

One aspect of our results supports the possibility of mating among closely related strains, as opposed to selfing alone, as contributing to the high levels of homogeneity observed for autosomal sequences. Sequences from the *qa* gene cluster exhibited the same general RFLP pattern as other autosomal sequences (*i.e.*, sibling *a* and *A* strains exhibited identical RFLP patterns, while substantial geographical polymorphism was evident among parental strains; results not presented). In *N. crassa*, and presumably also in *N. tetrasperma*, the *qa* gene cluster is tightly linked to the centromere, with no crossovers between the *qa* gene cluster and the centromere observed in several hundred *N. crassa* asci (CASE and GILES 1976). In the absence of crossing over, selfing alone should not produce the results observed here. Our results with *qa* sequences are compatible, however, with the alternative hypothesis that genetic homogeneity results from matings at the population level.

We currently have no information to support matings in nature between strains from different geographic locations. To the contrary, attempted matings between *N. tetrasperma* strains from diverse locations (laboratory outcrosses) very frequently result in sexual dysfunction, with abnormalities ranging from reduced ascospore viability to complete sterility (JACOBSON 1995). This fact, coupled with the observed suppressed recombination on the mating-type chromosome, suggests the possible accumulation of recessive mutations on that chromosome, with any given recessive allele sheltered by a functional allele on the sibling homologue. This hypothesis may require an explanation for the fact that it is typically possible to obtain *a* and *A* homokaryons capable of growing on minimal medium, which argues against an accumulation of recessive mutations lethal in the vegetative phase. Assuming that *within*-population matings occur among *N. tetrasperma* strains in nature, a scenario can be envisioned in which loci on the mating-type chromosome that are required for sexual reproduction or development (mating-type locus excluded) are targets for loss of one functional allele, whereas recessive alleles that prevent vegetative growth or asexual reproduction would be selected against in homokaryotic ascospores or conidia. It remains to be determined whether genes responsible for sexual dysfunction reside on the mating-type chromosome.

The possibility that sexual dysfunction results from combinations of recessive alleles is perhaps most plausible in instances where laboratory crosses abort early in sexual development, before ascospore formation. This explanation seems less credible in those instances, more frequently observed (JACOBSON 1995), where ascospores are produced but progeny are skewed away from self-fertile heterokaryons. One possibility is that this latter type of sexual dysfunction is in reality the manifestation of incompatible allele combinations at heterokaryon incompatibility loci. Other species of Neurospora, most notably *N. crassa*, are known to possess several allelic heterokaryon incompatibility loci that prevent the formation of stable heterokaryons between most wild-collected strains (MYLYK 1976; PERKINS 1988). As a natural heterokaryon, *N. tetrasperma* must in some manner avoid heterokaryon incompatibility. One pathway to this end would be to suppress the expression of a given heterokaryon incompatibility locus. This appears to be the case for the heterokaryon incompatibility function of the mating-type locus, which in *N. tetrasperma* is suppressed by the *tol* gene (JACOBSON 1992). An alternative pathway would be to maintain homogeneity at individual loci responsible for heterokaryon incompatibility (through selfing or otherwise). In this context, it is possible to speculate that the lack of variability observed in our study for sequences on autosomal chromosomes reflects selection to maintain allelic compatibility at heterokaryon incompatibility loci, rather than simply the cumulative, stochastic effects of selfing or inbreeding. This explanation would appear to imply for *N. tetrasperma* a strong hindrance to outcrossing in nature.

Relationship to sex chromosome evolution: In mammals, it is commonly assumed that sex chromosomes have evolved via a pathway that includes, first, a breakdown in recombination and, second, a degeneration of the chromosome that is responsible for the heterogametic condition (usually designated the *Y* chromosome; CHARLESWORTH 1978; RICE 1987a,b, 1994). The suppression of recombination along the length of the *N. tetrasperma* mating-type chromosome presents an interesting parallel with this proposed pathway. This parallel will be even stronger if it can be shown that suppression of recombination, together with pseudohomothallism, has led to the accumulation of certain nonfunctional alleles. Current theories proposed to explain the evolution of mammalian sex chromosomes are in some instances intrinsically linked to the existence of two different sexes, one being homogametic and the other heterogametic. More specifically, it is proposed that the great reduction in *X-Y* recombination permits the accumulation on *Y* chromosomes of genes that are of benefit to males but detrimental to females (sexually antagonistic genes), a theory that cannot be applied easily to *N. tetrasperma*. Degeneration of the *Y* chromosome has been attributed to Muller's ratchet and other forces that come into play due to that chromosome's essentially asexual nature (CHARLESWORTH 1978; RICE 1987a,b, 1994), a theory that may well be applicable to *N. tetrasperma*. *N. tetrasperma* could therefore prove valuable to the study of sex-chromosome evolution, in the sense that the development and testing of theories independent of the genetics of gender will be possible.

We thank DAVID PERKINS and NAMBOORI RAJU, Department of Biological Sciences, Stanford University, and ROBERT METZENBERG, Department of Biomolecular Chemistry, University of Wisconsin, Madison, for helpful comments on earlier versions of the manuscript. This research has been supported by grants to D.O.N. from the National Science Foundation (BSR-8415750, DMB-9022177) and to M.A.N. from the National Institutes of Health (GM-47374).

LITERATURE CITED

CASE, M. E., and N. H. GILES, 1976 Gene order in the *qa* gene cluster of *Neurospora crassa*. Mol. Gen. Genet. **147:** 83–89.

CENTOLA, M., and J. CARBON, 1994 Cloning and characterization of centromeric DNA from *Neurospora crassa*. Mol. Cell. Biol. **14:** 1510–1519.

CHARLESWORTH, B., 1978 Model for evolution of Y chromosomes and dosage compensation. Proc. Natl. Acad. Sci. USA **75:** 5618–5622.

DODGE, B. O., 1927 Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*. J. Agric. Res. **35:** 289–305.

FELSENSTEIN, J., 1993 PHYLIP (Phylogeny Inference Package) Version 3.5p.

GILES, N. H., M. E. CASE, J. BAUM, R. GEEVER, L. HUIET *et al.*, 1985 Gene organization and regulation in the *qa* (quinic acid) gene cluster of *Neurospora crassa*. Microbiol. Rev. **49:** 338–358.

GLASS, N. L., S. J. VOLLMER, C. STABEN, J. GROTELUESCHEN, R. L. METZENBERG *et al.*, 1988 DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. Science **241:** 570–573.

GLASS, N. L., J. GROTELUESCHEN and R. L. METZENBERG, 1990 *Neurospora crassa A* mating-type region. Proc. Natl. Acad. Sci. USA **87:** 4912–4916.

HOWE, H. B., 1963 Markers and centromere distances in *Neurospora tetrasperma*. Genetics **48:** 121–131.

HOWE, H. B., and P. HAYSMAN, 1966 Linkage group establishment in *Neurospora tetrasperma* by interspecific hybridization with *N. crassa*. Genetics **54:** 293–302.

JACOBSON, D. J., 1992 Control of mating type heterokaryon incompatibility by the *tol* gene in *Neurospora crassa* and *N. tetrasperma*. Genome **35:** 347–353.

JACOBSON, D. J., 1995 Sexual dysfunction associated with outcrossing in *Neurospora tetrasperma*, a pseudohomothallic ascomycete. Mycologia **87:** 604–617.

KERRIGAN, R. W., J. C. ROYER, L. M. BALLER, Y. KOHLI, P. A. HORGEN *et al.*, 1993 Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. Genetics **133:** 225–236.

LANGTON, F. A., and T. J. ELLIOTT, 1980 Genetics of secondarily homothallic Basidiomycetes. Heredity **45:** 99–106.

METZENBERG, R. L., and S. K. AHLGREN, 1973 Behaviour of *Neurospora tetrasperma* mating-type genes introgressed into *N. crassa*. Can. J. Genet. Cytol. **15:** 571–576.

METZENBERG, R. L., and N. L. GLASS, 1990 Mating type and mating strategies in *Neurospora*. BioEssays **12:** 53–59.

METZENBERG, R. L., and J. GROTELUESCHEN, 1993 Restriction polymorphism maps of *Neurospora crassa:* update. Fungal Genet. Newsl. **40:** 130–138.

METZENBERG, R. L., J. N. STEVENS, E. U. SELKER and E. MORZYCKA-WROBLEWSKA, 1984 A method for finding the genetic map position of cloned DNA fragments. Neurospora Newsl. **31:** 35–39.

MYLYK, O. M., 1976 Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora crassa*. Genetics **83:** 275–284.

PERKINS, D. D., 1985 Aspects of the *Neurospora* genome, pp. 277–294 in *Molecular Genetics of Filamentous Fungi*, edited by W. E. TIMBERLAKE. A. R. Liss, Inc., New York.

PERKINS, D. D., 1988 Main features of vegetative incompatibility in *Neurospora*. Fungal Genet. Newsl. **35:** 44–46.

PERKINS, D. D., 1992 *Neurospora crassa* genetic maps. Fungal Genet. Newsl. **39:** 61–70.

PERKINS, D. D., and B. C. TURNER, 1988 *Neurospora* from natural populations: toward population biology of a haploid eukaryote. Exp. Mycol. **12:** 91–131.

PERKINS, D. D., B. C. TURNER and E. G. BARRY, 1976 Strains of *Neurospora* collected from nature. Evolution **30:** 281–313.

PERKINS, D. D., A. RADFORD, D. NEWMEYER and M. BJORKMAN, 1982 Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. **46:** 426–570.

RAJU, N. B., 1980 Meiosis and ascospore genesis in *Neurospora*. Eur. J. Cell Biol. **23:** 208–223.

RAJU, N. B., 1992a Functional heterothallism resulting from homokaryotic conidia and ascospores in *Neurospora tetrasperma*. Mycol. Res. **96:** 103–116.

RAJU, N. B., 1992b Genetic control of the sexual cycle in *Neurospora*. Mycol. Res. **96:** 241–262.

RAJU, N. B., and D. D. PERKINS, 1994 Diverse programs of ascus development in pseudohomothallic species of *Neurospora, Gelasinospora,* and *Podospora*. Dev. Genet. **15:** 104–118.

RICE, W. R., 1987a The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. Evolution **41:** 911–914.

RICE, W. R., 1987b Genetic hitchhiking and the evolution of reduced genetic activity of the *Y* sex chromosome. Genetics **116:** 161–167.

RICE, W. R., 1994 Degeneration of a nonrecombining chromosome. Science **263:** 230–232.

SHEAR, C. L., and B. O. DODGE, 1927 Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. J. Agric. Res. **34:** 1019–1042.

STABEN, C., and C. YANOFSKY, 1990 *Neurospora crassa a* mating-type region. Proc. Natl. Acad. Sci. USA **87:** 4917–4921.

STEVENS, J. N., and R. L. METZENBERG, 1982 Preparing *Neurospora* DNA: some improvements. Neurospora Newsl. **29:** 27–28.

SUMMERBELL, R. C., A. J. CASTLE, P. A. HORGEN and J. B. ANDERSON, 1989 Inheritance of restriction fragment length polymorphisms in *Agaricus brunnescens*. Genetics **123:** 293–300.

SWOFFORD, D. L., 1993 PAUP: Phylogenetic Analysis Using Parsimony. Illinois Natural History Survey, Champaign, IL.

SWOFFORD, D. L., and G. J. OLSEN, 1990 Phylogeny reconstruction, pp. 411–501 in *Molecular Systematics*, edited by D. M. HILLIS and C. MORITZ. Sinauer Associates, Inc., Sunderland, MA.

VOLLMER, S. J., and C. YANOFSKY, 1986 Efficient cloning of genes of *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **83:** 4869–4873.

Communicating editor: R. H. DAVIS

APPENDIX

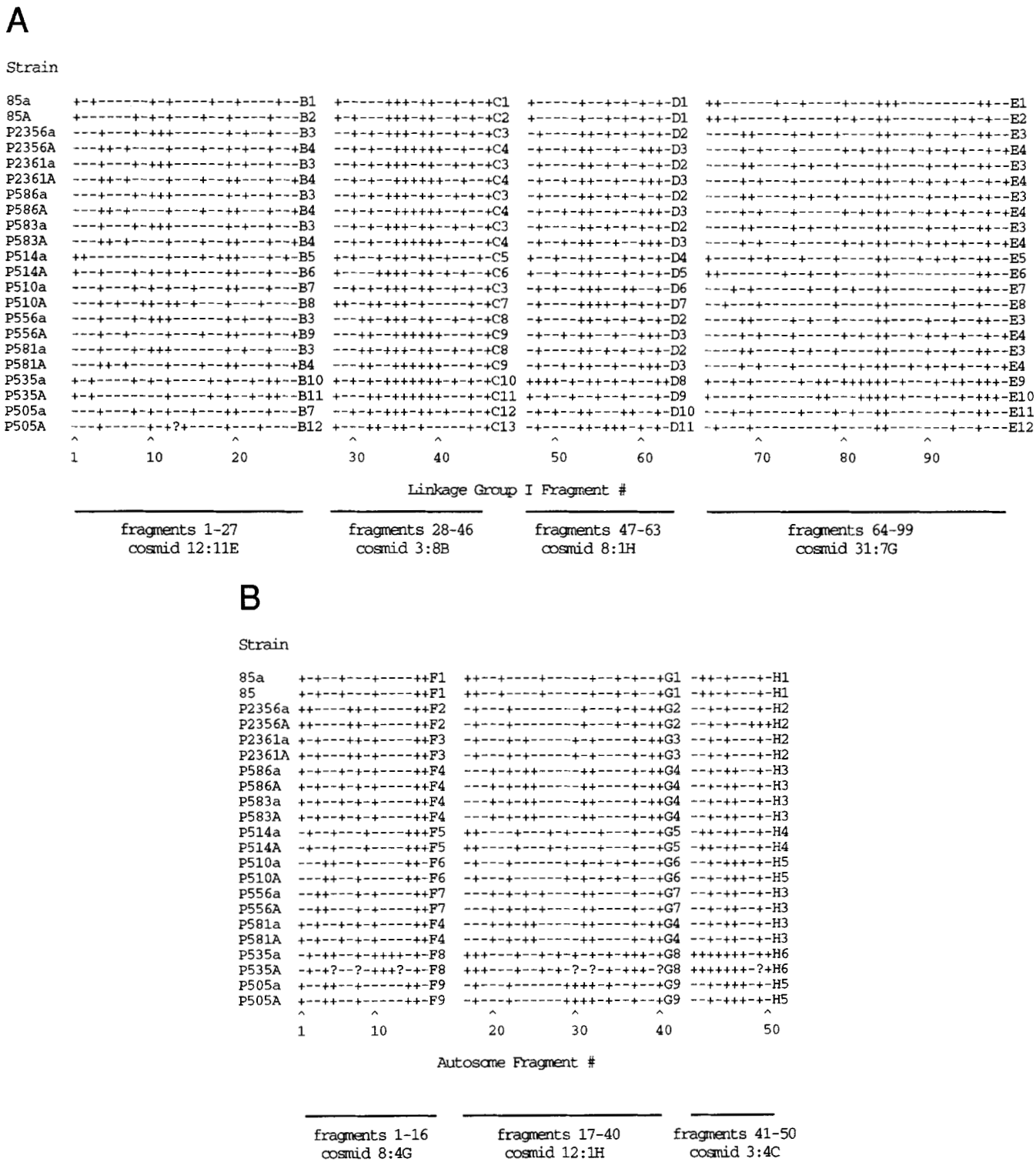

FIGURE A1.—Restriction-endonuclease fragment scores (+, present; −, absent) employed in tree-building and haplotype analyses. The scores are grouped in terms of the cosmid clones by which they were revealed. Haplotype designations are also presented. (A) Linkage-group I (mating-type chromosome) scores. (B) Autosome scores. Fragments are listed by size in Table 2. Several small and/or faintly hybridizing fragments were scored from autoradiograms but are not visible on the photographs presented in Figure 3.

TABLE A1

Scored restriction fragments in kilobase pairs

Mating-type chromosome				Autosomes[a]		
Cosmid 12:11E Characters 1–27	Cosmid 3:8B Characters 28–46	Cosmid 8:1H Characters 47–63	Cosmid 31:7G Characters 64–99	Cosmid 8:4G Characters 1–16	Cosmid 12:1H Characters 17–40	Cosmid 3:4C Characters 41–50
12.1	20.1	14.5	13.0	19.2	15.4	20.3
11.3	18.8	9.4	9.4	14.1	12.5	18.8
8.9	18.1	7.8	9.0	12.9	11.9	16.4
8.4	17.7	7.2	8.7	11.9	10.6	15.1
7.9	15.7	6.6	8.4	10.8	9.9	13.1
7.7	14.4	6.3	8.1	10.6	9.8	11.1
6.4	12.8	6.1	7.7	10.5	8.4	10.1
6.2	11.5	5.5	7.5	8.5	8.2	8.7
6.0	10.3	4.9	7.4	8.0	6.4	4.6
5.2	9.7	4.1	6.8	7.7	6.0	3.6
4.2	8.8	3.2	5.9	7.0	5.6	
3.7	8.1	3.1	5.3	6.7	5.4	
3.7	5.3	2.6	5.0	6.5	5.0	
3.6	2.0	2.4	4.3	4.1	4.8	
3.4	1.9	2.2	4.1	3.4	4.6	
3.4	1.6	1.4	4.0	2.9	4.1	
3.3	1.5	1.3	3.8		3.8	
3.1	1.3		3.7		3.2	
2.9	1.1		3.5		3.1	
2.7			3.3		3.0	
2.6			3.2		2.5	
2.5			3.1		2.1	
2.3			2.8		1.7	
2.2			2.7		1.4	
2.0			2.6			
1.8			2.3			
1.7			2.0			
			1.9			
			1.9			
			1.7			
			1.6			
			1.5			
			1.5			
			1.4			
			1.4			
			1.3			

Duplicate values in any given column represent fragments that were distinguishable on autoradiograms but that were the same size measured to the nearest 0.1 kbp.

[a] Cosmids 8:4G, 12:1H and 3:4C represent linkage groups II, IV and VII, respectively.