Curr Genet (1996) 29: 293–300

ORIGINAL PAPER

David M. Geiser · Michael L. Arnold
William E. Timberlake

Wild chromosomal variants in *Aspergillus nidulans*

Received: 8 March 1995/9 August 1995

Abstract Pulsed-field gel electrophoresis and a chromosome-specific cosmid DNA library were used to determine the karyotypes of wild-type *Aspergillus nidulans* isolates from around the world. Overall, little structural variation was found, with a few major exceptions. One isolate possessed a non-essential B-chromosome of about 1.0 million base pairs (mb). Another isolate had undergone a non-reciprocal translocation of about 1.6 mb of chromosome VI onto chromosome VIII. Other than these chromosomal differences, these isolates appeared phenotypically normal. To analyze its effects on meiosis, the translocation isolate was outcrossed with another wild-type derivative that had a "normal" electrophoretic karyotype. This cross produced a range of phenotypes, including duplicated progeny that had a "barren" phenotype similar to that described for *Neurospora* partial disomics. The duplication was somewhat vegetatively unstable. This is the first association of sterility with chromosomal duplication in *A. nidulans*.

Key words *Aspergillus* · Electrophoretic karyotype · B-chromosomes · Translocations

Introduction

Pulsed-field gel electrophoresis has uncovered a great deal of chromosomal polymorphism within and between fungal populations (Mills and McCluskey 1990; Kistler and Miao 1992), including both length polymorphisms and structural rearrangements (inversions, translocations). Consideration of chromosome polymorphism, particularly rearrangements, is essential in the mapping of fungal genomes (Wang et al. 1994). In fungi with a high frequency of chromosomal rearrangements, different patterns of genetic linkage may exist at the population level. Therefore, the map inferred for a single isolate cannot necessarily be applied to other isolates. Also, unlinked genetic markers are increasingly being used to infer levels of clonality in fungal populations (Tibayrenc et al. 1991). If karyotypes vary greatly among isolates in populations, different degrees of physical linkage may foil attempts to estimate clonal reproduction based on non-random associations of genetic markers. The effects of chromosomal variants on outcrossing are also important to consider. Some fungi that have been shown to harbor considerable natural variation, including *Aspergillus flavus* and *Aspergillus niger* (Keller et al. 1992; Swart et al. 1994), are being developed as molecular genetic systems. The relationship between karyotypic differences and sexual and parasexual outcrossing is important to consider when wild isolates are outcrossed to generate novel expression patterns of commercially useful compounds (Merrick 1975; Käfer 1977).

A. nidulans is a model system used in a variety of research areas, including those listed above. In previous work, we determined the electrophoretic karyotypes of members of the 20 known heterokaryon compatibility (hc) groups in the British *A. nidulans* population (Geiser et al. 1994). Sixteen representatives of different groups had karyotypes that were very similar to the "Glasgow" karyotype identified for strain FGSC4 (Brody and Carbon 1989), with six chromosomal bands representing the eight *A. nidulans* linkage groups. There was some size variation, most evident in chromosome V (harboring the ribosomal gene repeat), but only three apparent structural variants were found in the British

D.M. Geiser (✉)[1] · M.L. Arnold
Department of Genetics, University of Georgia, Athens, GA 30602, USA

W.E. Timberlake
Myco Pharmaceuticals, Inc., One Kendall Square, Bldg. 300, Cambridge, MA 02139, USA

[1] *Present address*: Department of Plant Biology, 111 Koshland Hall, University of California, Berkeley, CA 94720, USA

Communicated by O.C. Yoder

population: those in isolates M85, N89 and D34 (see Fig. 1 A).

It has been proposed that unbalanced chromosomal rearrangements are rare in *Neurospora* because of the deleterious effects of chromosomal duplications in progeny from crosses between strains with different arrangements. *Neurospora* partial diploids are usually partially or fully sterile ("barren", Perkins and Barry 1977), forming abnormal perithecia that contain few if any normal ascospores. In *Aspergillus*, infertility has not been associated with segmental chromosomal duplications (Käfer 1977; Perkins and Barry 1977; Selker 1990); however, different disomic and trisomic strains have a variety of distinctive abnormal phenotypes (Käfer and Upshall 1973), and segmental disomics have been shown to be vegetatively unstable and have abnormal morphology (Bainbridge and Roper 1966). Our understanding of the relationship between chromosomal rearrangements and phenotype currently does not extend beyond "Glasgow"-derived strains.

To ascertain if chromosomal variants are as rare outside of Great Britain, we have studied the electrophoretic karyotypes of nine additional *A. nidulans* isolated from around the world. We determined the origin of two of the structural variants from the British population by using a chromosome-specific cosmid DNA library (Brody et al. 1991), in which the chromosomal hybridization profile is known for each clone. Brody et al. (1991) hybridized gel-isolated chromosomes onto cosmid banks, producing chromosomal hybridization signatures for each cosmid in the library. By hybridizing the cosmid library with novel chromosomes from isolates with different karyotypes, we were able to infer the origin of novel chromosomes from the signatures produced by Brody et al. (1991). Then, we analyzed the effects of a wild non-reciprocal translocation on meiosis in an experimental cross. The results show an unusual correlation between a segmental duplication and fertility, very much like the "barren" phenotype described in *Neurospora*.

Materials and methods

Aspergillus strains, genetic manipulation and growth media. Sources of strains used in this study are listed in Table 1. Wild strains were examined using light, and in some cases electron, microscopy to ensure that they had the typical *A. nidulans*-type ascospore (Raper and Fennell 1965). *A. nidulans* isolates M85-1 and N89-2, kindly provided by Dr. R.B.G. Dales, were derived from their wild-type parents by UV mutagenesis. Isolate M85-1 has white conidia, most likely due to a mutation at the *w*A locus (Mayorga and Timberlake 1990), and isolate N89-2 has yellow conidia, most likely due to a mutation at the unlinked *y*A locus (O'Hara and Timberlake 1987). *A. nidulans* isolates M85-1 and N89-2 were crossed by co-streaking conidia on solid CM plates (Barratt et al. 1965) taping them shut, and placing them in a dark drawer. Cleistothecia were picked after 14 days from regions bearing both yellow and white conidiophores. Crossed cleistothecia were easily identified by the presence of green progeny (wA^+, yA^+). Fifty individual cleistothecia were rolled on 3% agar to remove conidia and Hülle cells, crushed in 100 µl of sterile water, and ascospores were counted twice with a haemocytometer. The ascospores were then plated onto solid CM at approximately 100 per plate; 34 progeny were randomly picked, pure cultured, and stored on silica gel.

Pulsed-field gel electrophoresis and Southern analysis. A. nidulans protoplasts were isolated and chromosomal DNA plugs were prepared as described previously (Brody and Carbon 1989; Geiser et al. 1994). *Saccharomyces cerevisiae* chromosome plugs were purchased from BioRad. Chromosomes were separated by using contour-clamped homogeneous field (CHEF; Chu et al. 1986) electrophoresis with a Bio-Rad CHEF DR-II unit in 0.8% Megarose gels (Clontech Laboratories; gel strength: > 2450 g/cm^2) at 47 V in 0.5 x TAE at 10 °C, with the following pulsing parameters (Geiser et al. 1994): 50-min pulses for 73 h; 45-min pulses for 18 h; 37-min pulses for 73 h. Samples were transferred to nylon membranes in the presence of 20 x SSC after acid depurination and alkaline denaturation (Geiser et al. 1994). The size estimates for the novel chromosomes present in isolates M85 and N89 were based on comparisons with *S. cerevisiae* chromosomes and with *A. nidulans* isolate FGSC4 (Brody and Carbon 1989).

Table 1 Sources of *A. nidulans* strains used in this study

Strain	Source	Isolation
M85	R.B.G. Dales	Soil, Pembroke, UK
N89	"	Soil, Cambridge, UK
M85-1	"	White-spored UVderivative of M85
N89-2	"	Yellow-spored UV derivative of N89
D34	"	*Collybia* sporophore, Birmingham, UK
FGSC4 Glasgow, UK[a]	Fungal Genetics Stock Center	
15737 (Quebec)	Caroline Badcock	Fax, Quebec, Canada
191525 (British Columbia)	"	Hay, British Columbia, Canada
Saudi Arabia	Martha Christensen	Soil, Saudi Arabia
Cincinnati	Judith Rhodes	University of Cincinnati Hospital
H995 (Venezuela)	Richard Hanlin	Soil, Venezuela
VA7-01 (Virginia 7)	David Geiser	Soil, Virginia
VA15-01 (Virginia 15)	"	"
NRRL 1079 (Texas 1)	Nancy Keller	Soil, Texas
SDG027-060	David Geiser	Progeny from M85-1 x N89-2 cross

[a] This isolate is often referred to as "Glasgow", but it appears to have been isolated from the air of a garden shed in Acomb, York, UK, by E. Yuill in 1937 (Yuill 1939).

Preparation of cosmid and plasmid DNA and gel-isolation and radio-labelling of DNA. The 1.9-megabase chromosome from isolate N89-2 and the 1.0-megabase chromosome from isolate M85 were gel-isolated by using β-agarase (New England Biolabs) according to the manufacturer's instructions. The chromosomes were separated for gel-isolation under the above conditions, except with a 1.0% low-melting-point agarose gel, and separation was performed at 60 V with 20-min pulses of 90 h. The plasmid pOGO4, containing the *pen*N gene from the *npe*A gene cluster on a 4.2-kb *Hin*dIII fragment, was a gift from the Eli Lilly Company. Chromosomal, cosmid, and plasmid DNAs were radioactively labelled with α-^{32}PdCTP by a random priming method (Promega). Plasmid and cosmid DNAs were isolated as described (Geiser et al. 1994). Labelled DNA was hybridized to membrane-bound DNA as described by Geiser et al. (1994).

Identification of chromosomal origins using the chromosome-specific cosmid library. Brody et al. (1991) hybridized CHEF-separated, gel-isolated *A. nidulans* chromosomes onto cosmid banks, producing hybridization signatures for each cosmid clone. The 1.9-megabase chromosome from isolate N89-2 and the 1.0-megabase chromosome from isolate M85 were probed onto the same cosmid banks, and strong positive signals were noted. The chromosomal signatures from Brody et al. (1991) were then used to infer the origins of the novel chromosomes from isolates N89-2 and M85.

Results

Low levels of karyotype variation in A. nidulans

The low level of karyotypic variation observed in the British population was also evident in *A. nidulans* isolates from elsewhere around the world (Fig. 1B). Nine isolates showed some differences in chromosome size, but all shared the six-band pattern with the British strains. The isolate from Texas (NRRL 1079) is the most variant, with all six chromosomal bands being larger than in other *A. nidulans* isolates.

Determination that the Cambridge karyotype was due to a non-reciprocal translocation

Figure 1 A shows that isolate N89 ("Cambridge") possesses an approximately 1.9-megabase chromosomal band not present in the Glasgow karyotype, and the chromosome-VIII band is larger in Cambridge than in Glasgow. Otherwise, these two isolates appear to possess the same chromosomal bands. We determined the origin of the Cambridge karyotype by determining the chromosomal identity of the approximately 1.9-megabase chromosome. We gel-isolated the chromosomal band, radioactively labelled it and probed it onto membranes containing clones from an *A. nidulans* chromosome-specific cosmid library, in which the chromosomal origin of each clone was known (Brody et al. 1991). Of the more than 3700 clones bound to the membranes, there were 222 strong positive signals that were chosen. Of the 222 clones corresponding to these signals, 180 were chromosome-VI positive, and 94 hybridized solely with chromosome VI from the work of Brody et al. (1991) (Table 2); 14 clones inferred to belong to other chromosomes by Brody et al. (1991) also showed hybridization to the approximately 1.9-megabase chromosome. Six of these clones hybridized solely with chromosome VIII, the remaining eight with chromosomes I, II, III, IV and V (Brody et al. 1991).

Cosmids that were positive with the *orl*A (*o*smotically-*r*emediated *l*ysis; Borgia and Dodge 1992) gene on chromosome VI, which maps to the right of the

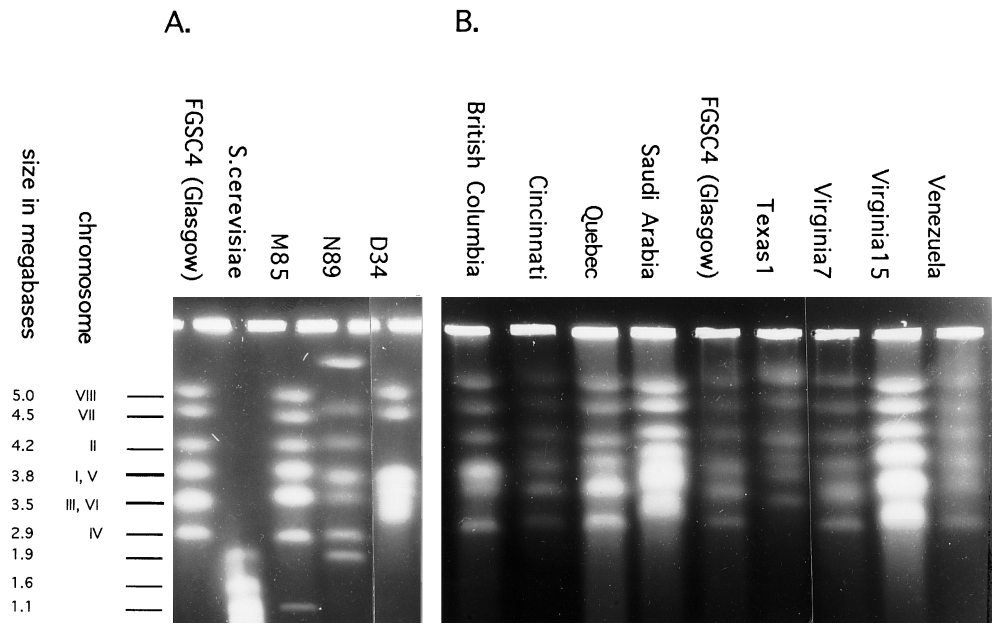

Fig. 1A, B CHEF-separated chromosomes from wild *A. nidulans* isolates. Size markers are based on those estimated for the FGSC4 wild-type (Brody and Carbon 1989) and for *S. cerevisiae* isolate YPH80 (GIBCO/BRL Co.) **A** three British isolates with unusual electrophoretic karyotypes, compared with the "Glasgow" isolate FGSC4 and *Saccharomyces cerevisiae* chromosomes. **B** isolates from outside Great Britain, compared with the Glasgow isolate

Table 2 Hybridization of novel chromosomes from isolates N89 and M85 (Fig. 1 A) with an *A. nidulans* chromosome-specific cosmid DNA library. The novel chromosomes in isolates N89 and M85 were gel-isolated and probed onto the chromosome-specific cosmid DNA library (Brody et al. 1991). "T" (total) rows represent the number of clones that hybridized to each *A. nidulans* chromosome as analyzed by Brody et al. (1991), which also hybridize with the novel approximately 1.9-mb chromosome in isolate N89, or the novel approximately 1.0-mb chromosome in isolate M85. "S" rows represent "S" (single-copy) clones, clones that hybridized to single *A. nidulans* chromosomes in the analysis of Brody et al. (1991). "S" rows represent a subset of the "T" rows. Cosmids not shown to hybridize to any chromosome by Brody et al. (1991) are categorized under "none". N is the total number of cosmids represented in each row

Isolate	Chromosome									
	I	II	III	IV	V	VI	VII	VIII	None	N
N89 T	46	50	73	65	75	180	38	48	11	222
N89 S	1	1	1	3	2	94	0	6	–	108
M85 T	57	90	120	115	77	105	70	74	1	137
M85 S	0	1	1	5	2	0	0	0	–	9

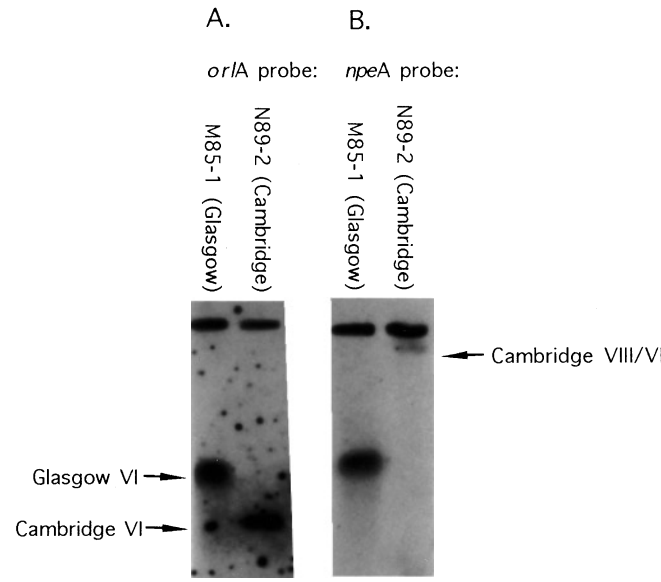

Fig. 2A, B Location of genetic markers on isolates M85-1, N89-2 and in their progeny. **A** hybridization of the *orl*A cosmid with CHEF-separated chromosomes from isolates M85-1 and N89-2. **B** Hybridization of the *npe*A plasmid with M85-1 and N89-2 chromosomes

centromere, were also positive with the approximately 1.9-megabase chromosome. Cosmids that hybridized to the pOGO4 plasmid, which contains DNA cloned from the distal-most locus mapped on the right arm of chromosome VI (*npe*A; Cole et al. 1976), were not positive with this chromosome. A cosmid containing the *orl*A gene was radioactively labelled and hybridized to CHEF-separated chromosomes from the Glasgow and Cambridge karyotypes (Fig. 2 A). In the Glasgow karyotype, the normal position of chromosome VI hybridizes, whereas in the Cambridge karyotype, the approximately 1.9-megabase chromosome hybridizes, with no Glasgow-sized copy of chromosome VI hybridizing. CHEF-separated Glasgow and Cambridge chromosomes were then hybridized with radioactively-labelled plasmid containing cloned DNA from the *npe*A locus. In the Glasgow karyotype the normal chromosome-VI position showed a signal, while only the large chromosome-VIII band in the Cambridge karyotype was positive (Fig. 2 B). From these hybridizations, we conclude that about 1.6 megabases on the right arm of chromosome VI had been non-reciprocally translocated onto chromosome VIII, leaving an approximately 1.9-megabase remnant of chromosome VI, as diagrammed in Fig. 3. A 3.5-megabase band remains in the Cambridge karyotype because chromosomes III and VI were nearly the same size.

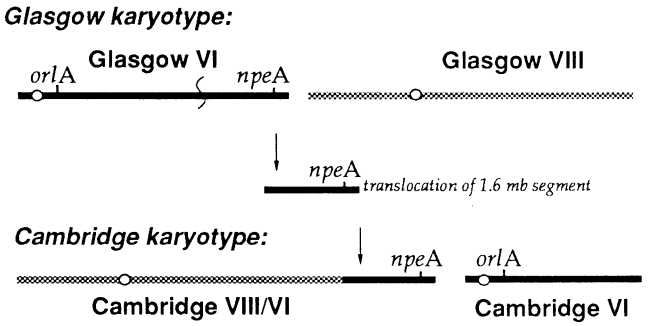

Fig. 3 Diagrammatic representation of the non-reciprocal translocation of 1.6 megabases of chromosome VI onto chromosome VIII, producing the Cambridge karyotype. The map locations of the *orl*A and *npe*A loci are shown (Clutterbuck 1992). The attachment of the translocated segment is depicted as telomeric, but it may have been insertional

Determination that the approximately 1.0-megabase chromosome in isolate M85 is a non-essential "B-chromosome"

Figure 1 A shows that isolate M85 possesses an approximately 1.0-megabase chromosome band in addition to the six bands present in most isolates. To determine whether this chromosome was also generated by a rearrangement, it was gel-isolated and probed onto the same chromosome-specific cosmid DNA library. One-hundred and thirty seven strong positive hybridization signals were identified, only nine of which represented cosmids that hybridized with single chromosomes in the previous determination of chromosome-specificity (Table 2). These nine "single-copy" cosmids represented four different chromosomes. When probed onto Southern blots of CHEF-separated chromosomes from ten different wild-type isolates, the

chromosome showed equally weak homology to all six chromosomal bands in all isolates (data not shown). Furthermore, a white-spored UV-derivative isolate of this strain (M85-1) lacks this chromosome, yet it grew normally on minimal medium (MM, Barratt et al. 1965) and appeared fully self-fertile.

Effects of crossing strains possessing Cambridge and Glasgow karyotypes on meiotic progeny

The Cambridge karyotype does not appear to affect the self-fertility of the strain possessing it. It grows normally both on minimal and rich media, and sporulates well. To look at the effect of the translocation on the isolate's ability to outcross, we crossed a white-spored isolate with a Glasgow karyotype (strain M85-1) with a yellow-spored isolate derived from the Cambridge isolate (strain N89-2). The parents of these strains were found to be nearly identical in a previous RFLP study (Geiser et al. 1994). Out of 50 cleistothecia picked, 37 were self-fertilized from the Glasgow-karyotype parent (producing white progeny only), two were self-fertilized from the Cambridge parent (yellow progeny only), two were biparental non-recombinants ("twins", white and yellow progeny but no green), and nine were outcrossed (white, yellow and green progeny); 34 green, yellow and white progeny were chosen from different crossed cleistothecia for further analysis, streaked to a single colony and stored. There was nothing unusual noted in the contents of outcrossed cleistothecia, and they contained ascospore counts similar to selfed cleistothecia.

A variety of phenotypes occurred in the outcrossed progeny. Variation was evident in colony shape, the quantity of cleistothecial development, the quantity of conidial development, and in the production of aerial hyphae. The most striking phenotype in the progeny was the production of "barren" selfed cleistothecia in 13/34 of the progeny chosen. These cleistothecia contained very few, if any, normal-looking ascospores. Instead, they contained mostly irregularly shaped ascospore-colored debris, approximately the size of an ascus, and smaller, ascospore-sized fragments of debris (Fig. 4). We use the term "barren" to describe these cleistothecia, following Raju and Perkins (1978) and Perkins (1994), who proposed the term to describe *Neurospora* perithecia containing few, or no, normal ascospores. In addition to the phenotype associated with meiotic reproduction, barren progeny conidiated poorly compared to their parents, and grew more slowly than their self-fertile siblings.

CHEF analysis of the progeny from the Glasgow x Cambridge cross

Random segregation of the translocated chromosome-VI segment was expected to produce four karyotypic classes (Fig. 5), including the two parental classes. CHEF analysis of the progeny showed three of the four possible karyotypes to be present. The duplicated, non-parental karyotype was present in all of the strains that had the barren phenotype described earlier. Each of these progeny possesses the large VI/VIII fusion product from the Cambridge translocation, but do not possess the approximately 1.9-megabase chromosome-VI product (Fig. 6 A). Hybridization of CHEF-separated chromosomes from these isolates with DNA from the *npe*A locus showed that two chromosomal bands are positive: both the VI/VIII translocation product and a band in the Glasgow chromosome-VI position (Fig. 6 B). These isolates are duplicated (partially disomic) for the translocated approximately 1.6-megabase chromosome-VI segment. The fourth expected class, which would be deleted for the translocated segment, was not observed.

Stability of the duplication in the infertile progeny

Bainbridge and Roper (1966) identified "crinkled" (*cr*) progeny from a cross between a laboratory derived isolate and another laboratory derived isolate with a non-reciprocal translocation of chromosome III onto

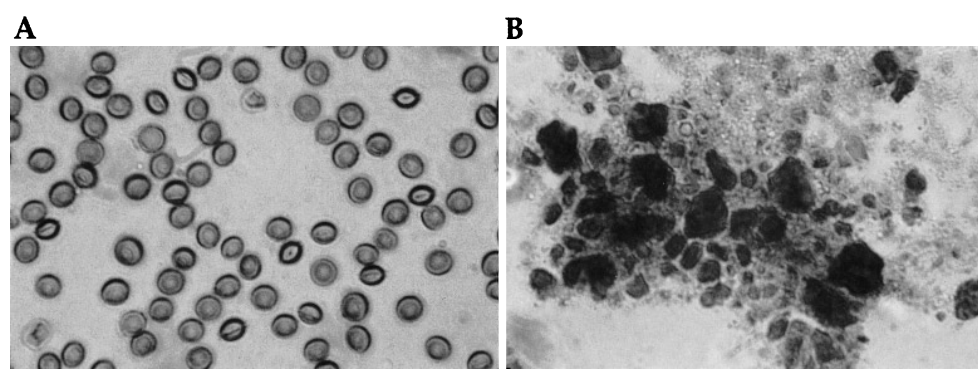

Fig. 4 Cleistothecium contents from **A** a Cambridge-karyotype progeny (strain SDG044) and **B** a progeny with the "barren" phenotype (strain SDG030). Both micrographs were taken using Nomarski optics and a 100 x objective under oil immersion

chromosome VIII. These progeny had a distinctive vegetatively unstable colony morphology, producing wild-type sectors at a frequency of about 50%. The *cr* phenotype was found to be associated with the segregant class duplicated for the translocated chromosome-III segment (Bainbridge and Roper 1966). We determined whether the same sort of instability occurred in the duplicated progeny of our "wild-type" Glasgow x Cambridge cross, without auxotrophic selection. Ten partially disomic progeny were point-inoculated onto two CM plates per isolate, and only one normal looking sector was found from the 20 plates. The sector was picked, single-colony purified, and its electrophoretic karyotype was determined. The derivative sector was found to have reverted to a Cambridge karyotype (data not shown). The reverted strain produced normal selfed ascospores.

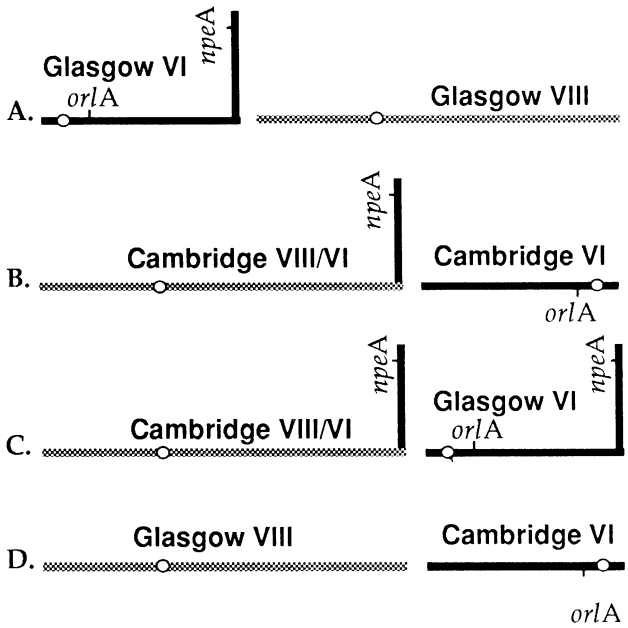

Fig. 5A–D Schematic diagram of four predicted karyotype classes in the progeny of the M85-1/N89-2 cross. **A** the "Glasgow" karyotype present in the M85-1 parent; **B** the "Cambridge" karyotype present in the N89-2 parent; **C** A type duplicated for the translocated segment observed in infertile progeny; **D** A type deleted for the translocated segment that was not observed in the progeny

Discussion

There was little apparent size variation in chromosomes from *A. nidulans* isolates from Great Britain (Geiser et al. 1994) and elsewhere. The Texas isolate was the most variant, all eight of its chromosomes being larger than in the Glasgow type. DNA sequence and RFLP evidence show this isolate to be more closely related to *Aspergillus rugulosus* than to other isolates of *A. nidulans*, although its ascospores have typical *A. nidulans* morphology (D.M. Geiser, Charles W. Mims, M.L. Arnold and W.E. Timberlake, unpublished data). Many fungi that contain a great deal of chromosomal-size variation, such as *Candida albicans* (Thrash-Bingham and Gorman 1992) and *Leptosphaeria maculans* (Plummer and Howlett 1993), have smaller chromosomes than *A. nidulans*. Size differences of the same magnitude found in these fungi would be less obvious in *A. nidulans* than in species with small

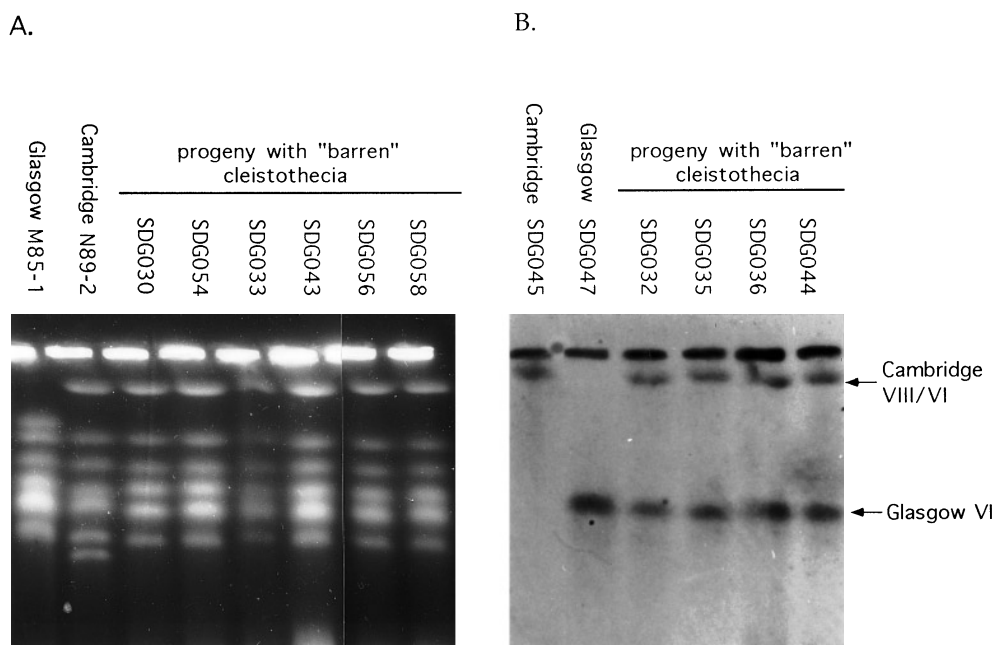

Fig. 6 **A** CHEF-separated chromosomes from six barren progeny from cross B, compared to the M85-1 and N89-2 parents. **B** hybridization of the *npe*A plasmid with CHEF-separated chromosomes from cross-B progeny

chromosomes. However, we have found that *Aspergillus fumigatus*, a species that is not known to produce ascospores, harbors a great deal of chromosomal variation within a population, although the sources of the variation (size differences vs rearrangements) have not been determined (D.M. Geiser, M.L. Arnold and W.E. Timberlake, unpublished data). Similarly high levels of variation have been described in other strictly mitotic groups of *Aspergilli*: *Aspergillus* section *Flavi* (Keller et al. 1992), and *Aspergillus* section *Nigri* (Swart et al. 1994). Clearly, there is less variation in *A. nidulans* than has been observed in other fungal genera with similar chromosome sizes, such as *Nectria haematococca* (Miao et al. 1991), *Fusarium oxysporum* (Kistler and Miao 1992), and *Cladosporium fulvum* (Talbot et al. 1991).

Only three major structural variants are apparent in *A. nidulans* isolates from Great Britain and elsewhere (Geiser et al. 1994). The Cambridge karyotype seen in isolate N89 was generated by a non-reciprocal translocation of about 1.6 megabases of the right arm of chromosome VI onto chromosome VIII. A very small region of chromosome VIII may have been transferred to chromosome VI in the rearrangement, since six single-copy cosmids from chromosome VIII hybridized to the approximately 1.9-megabase chromosome in isolate N89 (compared to 94 single-copy cosmids from chromosome VI). However, eight additional single-copy cosmids from chromosome I, II, III, IV and V also hybridized with the approximately 1.9-megabase chromosome. Much of this is most likely noise in the data set, perhaps reflecting differences between isolate N89 and the FGSC4 wild-type isolate used to make the chromosome-specific library (Brody et al. 1991).

A closely related strain, M85, possesses a chromosome of approximately 1.0 megabases that is absent in a derivative strain. The chromosome was probably lost due to the UV mutagenesis used to generate the white-spored mutation in isolate M85-1. Other than the white-spored mutation, which is presumably associated with the *w*A locus on chromosome II, there is no phenotype apparent in the derivative strain lacking the B-chromosome. Furthermore, the approximately 1.0-mb chromosome appears to contain little single-copy DNA, as 128/137 (93.4%) cosmids hybridizing with the approximately 1.0-mb chromosome hybridized with more than one *A. nidulans* chromosome. On average, cosmids hybridizing with the novel approximately 1.0-mb chromosome hybridized with 5.1 of the 8 *A. nidulans* chromosomes in the original determination of chromosome specificity (Brody et al. 1991). In comparison, the entire library consists of only 35.6% clones that hybridize to more than one chromosome (Brody et al. 1991). We propose that this chromosome is a non-essential B-chromosome. B-chromosomes are common in fungi (Kistler and Miao 1992), but this is the first reported for *Aspergillus*. A B-chromosome in the plant pathogen *Nectria haematococca* possesses a gene necessary for the detoxification of a host antifungal compound (Miao et al. 1991); however no phenotype is associated with the loss of B-chromosomes in *Magnaporthe grisea* (Valent and Chumley 1991) or *Cochliobolus heterostrophus* (Tzeng et al. 1992). The M85 B-chromosome is about the same size as the smallest observed in all *Aspergillus* species analyzed thus far, including *A. nidulans*, *A. rugulosus*, *A. navahoensis*, *A. fumigatus*, and members of the *Aspergillus* sections *Flavi* and *Nigri* (Keller et al. 1992; Geiser et al. 1994; Swart et al. 1994; Geiser, Arnold and Timberlake, unpublished data), raising the question of whether approximately 1.0 metabases is the minimum size for a stable chromosome in *Aspergillus*. The third structurally variant karyotype in isolate D34, which was not analyzed further, involves changes in size for both chromosomes II and IV and is most likely due to some sort of translocation.

The isolate harboring the Cambridge translocation is phenotypically normal, growing and sporulating well, and appears fully self- and cross-fertile. When crossed to an isolate with a Glasgow karyotype, normal outcrossed cleistothecia are formed. However, progeny are produced that are partially disomic for chromosome VI. Analysis of these progeny shows that they produce barren selfed cleistothecia that give rise to few or no normal ascospores, similar to those produced by crosses of duplicated *Neurospora* strains (Raju and Perkins 1977). To our knowledge, this is the first observation of sterility associated with a chromosomal duplication in *Aspergillus* (Käfer 1977; Perkins and Barry 1977). A similar barren phenotype has been observed in *aps*B (*a*nucleate *p*rimary *s*terigmata) mutants (Clutterbuck 1994), and diploid strains of *A. nidulans* show some similar debris in aborted cleistothecia. The unusual sterility found in these partial disomics may be specific to this particular segmental duplication, or it may be related to this particular non-isogenic cross. Because all of the barren progeny were observed to be partially disomic, it is clear that the barren phenotype is caused by the duplication. One sector was isolated from a partially disomic isolate that had reverted to the Cambridge karyotype. Bainbridge and Roper (1966) reported that partial chromosomal duplications produced in laboratory strains of *A. nidulans* are vegetatively unstable. The duplicated segments are presumably lost through a process termed mitotic nonconformity, where a nucleus lacking the duplication is produced during vegetative growth. The resulting nucleus is at a growth advantage, and produces a sector in the colony. The presence of only a single revertant sector suggests that this segmental duplication may be more stable than that observed by Bainbridge and Roper (1966). However, the partial VI-disomics produced in this work lack auxotrophic markers and were tested on a rich medium, which may increase stability. It has been proposed that chromosomal duplications confer sterility in *Neurospora* due to repeat-induced

point mutation (RIP) (Selker 1990). RIP has not been identified in *A. nidulans* (Selker 1990), so either the barren phenotype associated with the partial disomy is due to some other process, or there is an as yet unidentified RIP system in *Aspergillus*.

Figure 5 shows the expected effects of meiosis between the Cambridge and Glasgow karyotypes. First, full complementary pairing is expected, so crossing-over and segregation should not produce any invalid (dicentric, acentric) chromosomes. Random segregation is expected to produce four karyotypic classes: A, the "Glasgow" class; B, the "Cambridge" class; C, a partially disomic class that contains a duplication of the translocated segment; and D, a class deleted for the translocated segment. The first three classes were observed in the progeny of the M85-1 x N89-2 cross, but the deleted class was not. It is likely that such progeny, deleted for about 5% of their genome, are inviable.

Acknowledgements We thank Sarah Covert, Matthew Hare, Scott Hodges, Simon Emms, and Rolf Prade for helpful comments on the manuscript. We also thank those listed in Table 1 for providing strains. Special thanks to James Griffith for laboratory assistance, and David Perkins, Dick Dales, Brian Bainbridge, and Etta Käfer for advice and stimulating discussion. Karen Lillebjelke and Charles Mims provided assistance in the electron microscopy of some isolates. This work was supported by National Institutes of Health grant GM37886 to W.E.T. and National Science Foundation dissertation research grant DEB-9224224 to M.L.A., D.M.G. and W.E.T. D.M.G. was supported by National Institutes of Health predoctoral training grant A107373-01 from the National Institute of Allergies and Infectious Diseases.

References

Bainbridge BW, Roper JA (1966) J Gen Microbiol 42:417–424
Barratt RW, Johnson GB, Ogata NW (1965) Genetics 52:233–246
Borgia PT, Dodge CL (1992) J Bacteriol 174:377–383
Brody H, Carbon J (1989) Proc Natl Acad Sci USA 86:6260–6263
Brody H, Griffith J, Cutticchia AJ, Arnold J, Timberlake WE (1991) Nucleic Acids Res 19:3105–3109
Chu G, Vollrath D, Davis RW (1986) Science 234:1582–1585
Clutterbuck AJ (1992) *Aspergillus nidulans* genetic map. In: O'Brien SJ (ed) Genetic maps: book 3, lower eukaryotes. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
Clutterbuck AJ (1994) Microbiology UK 140:1169–1174
Cole DS, Holt G, McDonald KD (1976) J Gen Microbiol 96: 423–428
Geiser DM, Arnold ML, Timberlake WE (1994) Proc Natl Acad Sci USA 91:2349–2352
Käfer E (1977) Adv Genet 19:33–131
Käfer E, Upshall A (1973) J Hered 64:35–38
Keller NP, Cleveland TE, Bhatnagar D (1992) Curr Genet 21: 371–375
Kistler HC, Miao VPW (1992) Annu Rev Phytopathol 30:131–152
Mayorga ME, Timberlake WE (1990) Genetics 126:73–79
Merrick MJ (1975) J Gen Microbiol 91:278–286
Miao VPW, Covert SF, Van Etten HD (1991) Science 254: 1773–1776
Mills D, McCluskey K (1990) Mol Plant-Microbe Interact 3: 351–357
O'Hara EB, Timberlake WE (1987) Genetics 129:249–254
Perkins DD (1994) Mycologia 86:758–761
Perkins DD, Barry EG (1977) Adv Genet 19:133–285
Plummer KM, Howlett BJ (1993) Curr Genet 24:107–113
Raju NB, Perkins DD (1978) Can J Genet Cytol 20:41–59
Raper KB, Fennell DI (1965) The Genus *Aspergillus*. Williams & Wilkins, Baltimore, MD, USA
Selker EU (1990) Annu Rev Genet 24:579–613
Swart K, Debets AJM, Holub EF, Bos CJ, Hoekstra RF (1994) Physical karyotyping: genetic and taxonomic applications in *Aspergilli*. In: Powell KA, Renwick A and Peberdy JF (eds) The genus *Aspergillus*: from taxonomy and genetics to industrial applications. Plenum Press, New York, pp 233–240
Talbot NJ, Oliver RP, Coddington A (1991) Mol Gen Genet 229: 267–272
Thrash-Bingham C, Gorman JA (1992) Curr Genet 22:93–100
Tibayrenc M, Kjellberg F, Arnaud J, Oury B, Breniere SF, Darde M, Ayala FJ (1991) Proc Natl Acad Sci USA 88:5129–5133
Tzeng T, Lyngholm LK, Ford CF, Bronson CR (1992) Genetics 130: 81–96
Valent B, Chumley FG (1991) Annu Rev Phytopath 29:443–467
Wang Y, Prade RA, Griffith J, Timberlake WE, Arnold J (1994) Proc Natl Acad Sci USA 91:11094–11098
Yuill E (1939) J Botany 77:174–175