ENVIRONMENTAL AND GENETIC FACTORS AFFECTING CHROMOSOMAL INSTABILITY AT MITOSIS IN *ASPERGILLUS NIDULANS* AND THE IMPORTANCE OF CHROMOSOMAL INSTABILITY IN THE EVOLUTION OF DEVELOPMENTAL SYSTEMS[1]

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ABSTRACT: Previous work has shown that strains of *Aspergillus nidulans* with a chromosome segment in duplicate (one in normal position, one translocated to another chromosome) are unstable. Deletions occur from either duplicate segment. The present work has shown that the incidence of deletions is dependent upon the temperature of growth and on the particular chromosome segment which is duplicated. Irrespective of chromosomal duplication, deletions are generally confined to the translocated duplicate segment. At higher temperatures, it is found that specific deletions from either of two chromosomal duplications are most prevalent during a particular period of growth. When both duplications are together in a haploid, one duplication regulates the incidence of deletions from the other duplication, the incidence of deletions being a function of the size of the regulatory duplication. It also has been found that a triplicated chromosome segment in an *Aspergillus* diploid can induce genetic changes in chromosomes not linked to chromosomal triplication. It is argued that genetic instability-systems based on chromosomal imbalances or re-organizations, such as those found in *Aspergillus*, could have played a major role in the evolution of developmental systems.

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In the filamentous fungus *Aspergillus nidulans* (normally haploid), strains with a chromosome segment in duplicate (one segment in normal position, one translocated to another chromosome) are unstable at mitosis (Bainbridge and Roper, 1966; Nga and Roper, 1968). Duplication strains, which have a characteristic morphology (referred to as crinkled) and reduced linear growth rate, give sectors produced by nuclei which have undergone spontaneous deletions, of variable size, from one or other duplicate segment. (Mitotic crossing-over is not involved.) Sectors, referred to as improved sectors, can be scored unequivocally by their improved morphology and relative growth advantage, determined by nuclei more haploid in quantity--due to loss of genetic material--than those of the duplication parent. In suitably marked strains, loss of one or more dominant alleles gives improved sectors differing from the parent in conidial color and/or nutritional requirements. The approximate size of each deletion, and the segment involved, can be determined by genetic analysis or sometimes simply by the morphology, nutritional requirements and stability or instability of the sectors carrying it. The evidence has been that the deletions which occur in unstable duplication strains are provoked or induced by the chromosomal imbalance, i.e. by the duplication (Nga and Roper, 1969). All information indicated that a chromosomal

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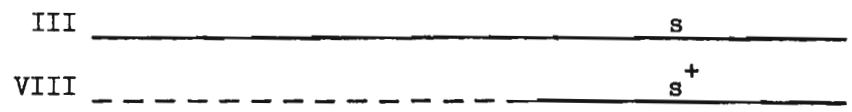
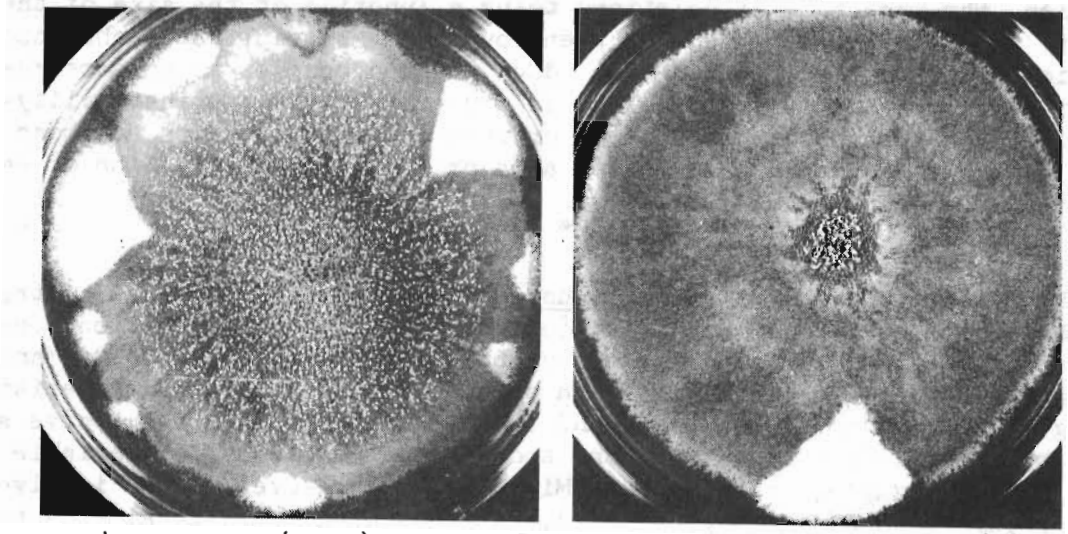


Fig. 1. A segment of chromosome III in duplicate (III duplication), one segment translocated to chromosome VIII. s is a recessive mutant allele which determines a requirement for thiosulphate. Cultures which carry this duplication are phenotypically thiosulphate-nonrequiring, i.e. $\frac{s^+}{}$.

Fig. 2. A segment of chromosome I in duplicate (I duplication), one segment translocated to chromosome II. The recessive mutant alleles determine; y, yellow conidia, as opposed to wild-type grass-green; ad and bi, requirement for adenine and biotine respectively. Cultures which carry only this duplication are phenotypically grass-green, and phenotypically adenine and biotin nonrequiring, i.e. $\frac{ad^+\ bi^+}{}$.



Figs. 3 and 4. Fig. 3 (left) shows a I duplication-strain colony with yellow and green sectors after incubation at 39.5°C for ten days. Fig. 4 (right) shows a I duplication-strain colony with a yellow sector after incubation at 36°C for ten days.

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imbalance rarely provoked deletions outside the particular segments involved in the imbalance. This was based on a study of diploid strains with an extra chromosome segment. In these diploid strains with the chromosomal imbalance, deletions were confined almost exclusively to the chromosome segments present in triplicate (Nga and Roper, 1969; Roper and Nga, 1969). When deletions were not confined to these segments present in triplicate (the triplication), deletions affected or included a chromosomal region adjoining the triplication--chromosomal imbalance--in question.

By means of genetic analyses and studies relating to percentages of various types of improved sectors, the present work has shown that most deletions occur from the translocated duplicate segment in duplication strains. Furthermore, it has been found that the overall frequency of deletions from a chromosome III duplication, henceforth called III duplication (see Fig. 1), is greatly enhanced

TABLE 1

Colonies of a crinkled, green[1] strain, Rg, carrying the III duplication and incubated for nine days at different temperatures

	Number of Rg colonies[2] (only one colony per complete-medium dish)	Mean diameter of non-mutant region[3] giving rise to improved mutant sectors	Mean number of improved sectors per colony[4]
Colonies at 42°C	60	4.2 cm	.86
Colonies at 36°C	40	3.3 cm	6.6
Colonies at 28°C	40	2.2 cm	7.0

[1]Duplication strains carrying the y^+ allele and the III duplication are in fact olive-green (i.e., have olive-green conidia). y^+ strains which do not carry the III duplication, or which carry a very small part of it, are grass-green in colour shade.

[2]Respective colonies arise from the respective conidial inocula placed in the respective centres of dishes of complete medium. This is the case for all colonies referred to in the other tables. Colonies (in this experiment) were obtained from the same sample of conidia.

[3]The colonial region of a colony which does not include mutant sectors.

[4]Using a statistical test described by Lehmann (1959), it was found that the means 6.6 and 7.0 are significantly different from the mean .86 at $P < .01$, but do not differ significantly from one another. Other studies showed that differential selection is not a contributing factor to the significant difference between particular sector-means (Lieber, 1972).

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by low temperatures (e.g., Table 1), while the overall frequency of deletions from a chromosome I duplication, henceforth called I duplication (see Fig. 2), is markedly enhanced by high temperatures (e.g., Table 2). A temperature of 39.5°C appears to enhance the overall frequency of deletions from the I duplication to the greatest extent. With regard to the deletion of particular regions of the translocated duplicate I segment, such as regions including the y^+ allele, the frequency of deletions encompassing particular alleles on the translocated duplicate I segment such as y^+, is apparently enhanced to the greatest extent by a temperature of 39.5°C (see Fig. 3; also note Fig. 4). With regard to regions on the non-translocated duplicate I segment, i.e. those including ad^+ and bi^+, an increase in temperature progressively enhances the frequency of deletions to which such regions are subject (e.g., Table 2). Moreover, by studying the frequencies of given types of improved sectors which occur during particular periods of growth, it was found that at respectively 39.5°C and 42°C specific regions of the I duplication are subject to far more deletions during a particular period of growth than during any other period, and at 42°C, a particular region of the III duplication is subject to far more deletions during a given period of growth than during any other period, thus suggesting that deletion of those regions at higher temperatures conforms to some type of internal programme (Lieber, 1972).

When the I duplication and the III duplication are together in a haploid, deletions from the intact III duplication generally precede deletions from particular sections of the I duplication. Furthermore, the III duplication can indirectly enhance to some (but not major) extent the frequency of deletions

TABLE 2

Colonies of a crinkled, grass-green strain, P, carrying the I duplication and grown at different temperatures

	Number of P colonies (only one colony per complete-medium dish; colonies obtained from same sample of conidia)	Mean diameter of non-mutant region giving rise to improved mutant sectors	% of green improved sectors found to be *ad bi* sectors[2]	Mean number of yellow sectors[3] per colony[4]	Mean number of improved sectors (including greens) per colony[4]
Colonies at 42°C for 12 days	38	6.7 cm	65%	3.2	3.7
Colonies at 39.5°C for 10 days	36	7.6 cm	25%	5.1	5.9
Colonies at 36°C for 10 days[1]	40	8.2 cm	8%	1.1	1.7
Colonies at 28°C for 12 days	40	8.3 cm	0%	.85	1.4

[1]By ten days incubation, circumferences of colonies at 36°C are close to the edge of dishes.

[2]Green *ad bi* sectors arise as a result of deletions which include *ad*$^+$ and *bi*$^+$ on the non-translocated duplicate I segment.

[3]All yellow sectors are improved; respective yellow sectors arise as a result of respective deletions which include the *y*$^+$ allele on the translocated duplicate I segment. All yellow sectors are phenotypically *ad bi*.

[4]Regarding yellow sectors, the means .85 and 1.1 are not significantly different but are each significantly different ($P < .01$) from the mean 5.1. At $P < .01$ the mean 3.2 is significantly different from the mean 5.1; moreover, at $P < .01$ the mean 3.2 is significantly different from the respective means 1.1 and .85. Regarding all improved sectors, the means 1.7 and 1.4 are not significantly different but are each significantly different ($P < .01$) from the mean 5.9; at $P < .01$ the mean 3.7 is significantly different from the means 5.9, 1.4, and 1.7. Studies have also shown that sectors are not subject to differential selection at different temperatures (Lieber, 1972).

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which include the *y*$^+$ allele on the I duplication. After the III duplication becomes considerably reduced in size as a result of the loss of chromosomal material from the translocated duplicate III segment, such a reduced III duplication can greatly enhance the frequency of deletions which include the *y*$^+$ allele on the I duplication (e.g. Table 3). In other words, a III duplication of greatly reduced size can promote far more deletions which include the *y*$^+$ allele on the I duplication than can the intact III duplication. The major increase in the deletional instability of the I duplication as promoted by the reduced III duplication is confined to the translocated duplicate I segment. The reduced III duplication can induce deletions from a section of the

TABLE 3

Single- and double-duplication strains incubated at 36°C

	Number of colonies (one colony per complete-medium dish)	Mean diameter of non-mutant region giving rise to mutant sectors	Mean number of yellow sectors per colony	Mean number of green-improved sectors per colony
Crinkled, grass-green strain, P, carrying the I duplication (incubated for 10 days)	40	8.2 cm	1.1	.6
Highly crinkled, olive-green haploid strain, R-P, carrying both the I duplication and the intact III duplication (incubated for 9 days). R-P was derived from a cross between Rg and P, but unlike Rg carries s on the translocated duplicate III segment	40	2.6 cm	2.9[1]	—
Crinkled, grass-green, haploid strain, h, carrying the I duplication and a III duplication of greatly reduced size (incubated for 9 days). Other than having the reduced III duplication, strain h has the same genotype as R-P and was obtained from highly improved, grass-green sectors produced by R-P through major deletions from the translocated duplicate III segment	61	5.7 cm	13.5	1.7

[1]Most yellow sectors produced by R-P arose from the highly improved grass-green sectors which emerged from the non-mutant region of R-P. Colonies (R-P) originally having the unaltered or intact III duplication gave rise at 36°C directly or indirectly (by means of green sectors or variants) on the average to far fewer yellow sectors than did colonies (h) at 36°C originally carrying a III duplication of greatly reduced size. This indicates that the originally reduced III duplication in h colonies gave rise to a situation which affected or greatly enhanced the frequency of those deletions including the y^+ on the translocated segment of the I duplication. Because it is indicated that a changed or altered III duplication can greatly change the yellow sectoring frequency and thus the frequency of deletions involving the y^+ allele on the I duplication, then it would follow that the region of the III duplication has some definite effect on the yellow sectoring frequency, and thereby upon the frequency of deletions which include the y^+ allele on the I duplication. In view of this, the fact that R-P colonies at 36°C gave rise on the average to

significantly more (at $P < .01$) yellow sectors than P colonies at 36°C would indicate that the originally unaltered III duplication determined the course which ultimately led to this significant increase in the frequency of deletions involving the y^+ allele on the I duplication. Each h colony would have in fact a reduced III duplication of independent origin as such colonies were derived from different improved sectors. In additional studies (Lieber, 1972), members of one group of h colonies all had the same reduced III duplication (i.e., the same in origin), while members of another group of h colonies all carried a reduced III duplication different in origin from that of the first group. Each group of h colonies still produced a high frequency of yellow sectors compared to a P colony control. Moreover, a reduced III duplication (originally present in one h colony) was still found in the F1 generation to enhance the frequency of deletions from the I duplication.

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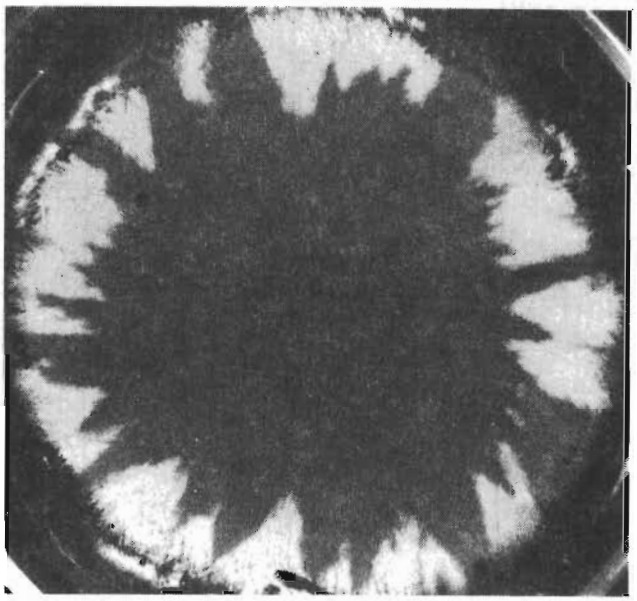

Fig. 5. A colony of "strain h" with yellow sectors at 36°C. Period of incubation was nine days.

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translocated duplicate I segment in accord with a temporal programme, as indicated by the fact that yellow sectors or variants commenced at points approximately equidistant from the respective centres of colonial-cultures of "strain h" (see Fig. 5). Moreover, as determined by genetic analysis the y^+ region of the I duplication is far more under the mutagenic influence of the reduced III duplication than is the bi region.

In cultures carrying single duplications, the underlying cause or regulation behind the induction of deletions from the single duplications in such cultures, as pointed out earlier, are the respective duplications themselves (Nga and Roper, 1969). In double-duplication cultures on the other hand, where the reduced III duplication is present, the I duplication largely loses its ability to direct or control deletions from itself; it appears that the reduced III duplication takes over this function. It is as if such control represents some kind of bond or tie between the two duplications. What is of interest here with regard to these duplications is that we have one genetic entity on the chromosomal level (as opposed to a single gene) programming or determining or directing an extremely high frequency of deletions from a second genetic entity on the chromosomal level, or from another point of view, we have one chromosomal imbalance causing or inducing a second chromosomal imbalance to become extremely unstable.

This information relating to a double-duplication strain and other information (Lieber, 1972) obtained through the study of diploids carrying a segment of chromosome III in tripilicate showed that a chromosomal imbalance either in the form of a duplication in a haploid or a triplicated chromosome segment in a diploid can cause mutations or deletions within chromosomal regions not linked to the chromosomal imbalance in question. In effect, a chromosomal imbalance can promote deletions or mutations outside itself within regions of the genome.

Such a duplication in a haploid and a triplication in a diploid can be regarded as complex genetic-mutators in that they can induce mutations in genetic regions not linked to the mutator in question. The reduced III duplication can be seen as a mutator which resulted from the instability of a much larger genetic complex, and a mutator which is able to provoke mutations in a regulated and specific manner. Mutators capable of indirectly inducing deletions of genetic material in a regulated and specific manner might be a necessary basis for development in various situations. In this connection, development in some cases is known to involve the selective elimination of genetic material (Waddington, 1956; Fischberg and Blackler, 1961; Beerman, 1966; Brown, 1969). On the other hand, some defects in development in higher organisms might be ascribed to genetic systems which can provoke deletions in a disorderly manner, either temporally or spatially. Some cases of neoplasia might have their basis in the deletions of genetic material indirectly induced by a genetic-complex, such as a very small triplication, after the cessation of development. Such a complex might very well have been derived after development through the environmental-conditioned instability of a larger genetic complex.

With regard to development, the double-duplication instability system in Aspergillus would be a very good example of an early type of developmental system which, in a programmed manner, was able to determine a type of differentiation through the induction of irreversible chromosomal changes in somatic (vegetative) tissue. This instability-system owes its ultimate origin to basic chromosomal reorganizations in the genome as exemplified by the existence of chromosome imbalances (or non-tandem duplications of genetic material) in a haploid genome. Many other types of chromosomal reorganization in other types of organisms may have been directly associated with--or may have resulted in--instability-systems, systems which could represent the first forms of developmental systems that effect types of differentiation through the induction of chromosomal or genetic changes. In maize, for example, chromosomal reorganizations ultimately resulted in instability-systems which promote mutations (and deletions) in a controlled manner, the incidence of which is affected by temperature (McClintock, 1951; McClintock, 1965). These systems in maize would also be other examples of early forms of developmental systems which effect types of differentiation through the induction of chromosomal or genetic changes.

Considering what has been said, then, particular chromosomal reorganizations in the distant past may very well have resulted in instability-systems, and various combinations of these instability-systems may have in turn evolved into complex developmental systems based upon the regulated induction of chromosomal or genetic changes. In other words, various instability-systems, or more ultimately, particular chromosomal reorganizations, may have provided in the distant past the basis for the evolution of complex types of developmental systems in which genetic changes of various types would play a major role. The very rate of such an evolution may have been greatly affected by the instability-systems in question, insofar as it is certain that such instability-systems would have also determined a very high degree of genetic and phenotypic variability over numerous generations. In general, instability-systems of various types could be seen as being factors of prime importance in evolution because of their capability for generating a very high degree of variability within populations, and because of their sensitivity, in various cases, to environmental factors, such as temperature.

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