

animals are responsible for the high frequency of the cyanogenic forms in those regions in which the molluscs occur. If this is true then we have clear evidence for the defensive role of cyanogenesis in natural populations. Experimental work¹⁵ shows that NaCl may have a differential effect on the root growth of cyanogenic and acyanogenic plants obtained from Porthdafarch. This could account for the low frequency of cyanogenic plants on the coastal sites. Because the habitat has been examin-

ed in such detail we feel confident that we can eliminate the other ecological variables from further consideration at Porthdafarch. This means that we have demonstrated the value of using a wide range of ecological techniques in this type of population genetics. We are fully aware, however, that what applies at Porthdafarch and on Holy Island is not necessarily true of other habitats.

15 R. J. Keymer and W. M. Ellis, in preparation.

Effects of ethidium bromide in diploid and duplication strains of *Aspergillus nidulans*¹

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Summary. Unstable duplication and diploid strains of *Aspergillus nidulans* were treated with ethidium bromide, and it was shown that this drug reduces the number of sectors produced by such strains. The mechanisms which could be responsible for the partial stabilization of the strains are discussed and it is suggested that a similar mechanism is responsible for the production of sectors in both strains. It is also suggested that ethidium bromide could be useful for the reduction of instability of industrial strains.

Strains of *Aspergillus nidulans* with a duplicate chromosome segment, one in a normal position and other translocated^{2,3}, are mitotically unstable. They produce sectors which arise from nuclei which have lost a variable part of one or other duplicate segment by an intra-chromosomal process. Such sectors are designated improved sectors^{4,5}. Duplication strains also produce, infrequently but regularly, sectors with deteriorate morphology, which were explained by new duplications arising within one or other duplication segment which can be transported all or in part to another site in the non-duplicated part of the genome⁶. Diploid strains of *A. nidulans* are also unstable producing sectors, which are originated from mitotic crossing-over or haploidization⁷⁻⁹. Environmental changes, as the presence of certain drugs in the culture medium, mutagenic agents or even genetic factors¹⁰⁻¹⁶, can effect sector

production, both in duplication and diploid strains. It can then be stated that diploid and duplication strains have a characteristic production of sectors which is maintained for each strain in the determined condition¹⁵. An attempt to elucidate the mechanisms involved in the production of sectors from a duplication strain is to compare the 2 systems: diploid and duplication, against the same drugs¹⁷. In the present work, ethidium bromide, an acridine which is known to bind to nucleic acids besides other biological effects (for review, see Levy et al.¹⁸), was used to compare its effects affecting the production of sectors from diploid and duplication strains submitted to the action of such drug.

Material and methods. The medium used was solid complete medium (CM) containing yeast extract, hydrolyzed casein, hydrolyzed nucleic acids, vitamins, etc.¹⁹ with 2%

Table 1. Sectors produced by duplication strain A in absence and in the presence of ethidium bromide

Ethidium bromide (µg/ml)	No. of dishes	Mean number of sectors per dish			Total
		Yellow	Green	Other*	
0.0	29	2.48	0.76	0.10	3.34
1.0	39	0.33	0.08	0.15	0.56
1.5	38	0.45	0.00	0.05	0.50

* includes deteriorated and heterokaryotic sectors.

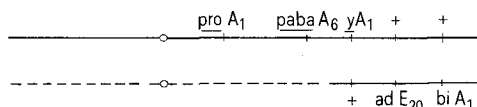
Table 2. Sectors produced by diploid strain biA1/MSE in absence and in the presence of ethidium bromide

Ethidium bromide (µg/ml)	No. of dishes	Mean number of sectors per dish		
		Macrosectors	Microsectors	Total
0.0	28	6.25	14.30	20.55
1.5	19	1.73	9.52	11.25
2.0	26	0.90	5.30	6.20

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agar added. Strains of *A. nidulans* used, all derived from Glasgow stocks, were: Diploid biA1/MSE strain, obtained from a heterokaryon between strain biA1 which requires biotin and the Master Strain E (MSE) of McCully and Forbes²⁰, through the Roper's method²¹, and Duplication strain A of Nga and Roper³ (figure). Ethidium bromide (EB) Sigma was added to CM in different concentrations all of which did not reduce, or only slightly reduced, the growth of the used strains. Conidia were inoculated in the centre of 10 cm diameter petri dishes containing CM without and with EB added, and after 7–8 days incubation at 37°C sectors were scored. Sectors from diploid strain were classified in 2 categories: macrosectors (with more than 20 conidiophores) and microsectors (with 20 or less conidiophores).

Results and discussion. Tables 1 and 2 give the number of sectors produced by duplication and diploid strains growing on medium with and without EB added. In both cases the number of sectors decreases in the presence of the drug which indicates a possible similarity between the mechanisms of sector production both in diploid and duplication strains. These results are in agreement with



Duplication strain A. Linkage groups I and II are shown by unbroken and broken lines respectively. Centromeres are designated by open circles. *ad E20*, *biA1*, *paba A6*, *pro A1* and *yA1* are respectively genes for adenine biotin, p-aminobenzoic acid, proline requirements and yellow conidia.

those obtained after treatment of diploid and duplication strains with 2 fungicides¹⁷ and with the results of diploids bearing duplications²³. At least in the case of the fungicide, 1,4-oxathiin, whose mode of action is inhibition of respiration, it is known that it reduces, in low concentrations and in the same pattern as shown by EB, the number of sectors from both diploid and duplication strains¹⁷. It is then possible that EB, which also acts on mitochondrial DNA, reduces indirectly the number of sectors due to a lack of energy as a consequence of inhibition of respiration. It is however also possible that EB can affect directly the haploidization and/or mitotic crossing over, due to its action upon DNA¹⁸ and on the repair mechanism²². Biochemical studies and also genetical studies, including the isolation of EB resistant mutants, could provide a better understanding of the action of the drug in relation to the number of sectors produced by both kinds of strains. In any case, it could be suggested that EB could be used to yield preservation in commercial fungal strains. Certain commercially useful strains may show an instability pattern due to low-yielding derivatives in the population of stored spores of the strain. Reduction of instability is in part achieved by environmental control or through a balanced lethal system²⁴, or even through point mutations¹⁵. EB could also be useful for this purpose when added to cultures of duplication or diploid strains.

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Sex chromosome polymorphism in *Oryzomys longicaudatus philippii* (Rodentia, Cricetidae)¹

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Summary. *Oryzomys longicaudatus philippii* has a diploid number $2N = 56$ ($N.F. = 70$). A polymorphism of X-chromosomes is described and a duplication as causal mechanism is postulated. The degree of chromosomal differences among the 4 karyological forms of *O. longicaudatus* and between those forms with *O. l. philippii*, enable us to postulate the level of full species for all of them.

Sex chromosome polymorphisms are not usual among rodents. They have been described in *Peromyscus*³, *Akodon*^{4,5}, *Mus*^{6,7}, *Spermophilus*⁸, *Neotoma*⁹, *Tatera*¹⁰, *Zygodontomys*^{11,12}, *Bandicota*¹³ and *Nesokia*¹⁴. In the present paper, a polymorphism of sex chromosomes of *Oryzomys longicaudatus philippii* is described for the first time, and the karyotype and idiogram of the species has been constructed. On the other hand, the systematic significance of the chromosomal variants of *O. longicaudatus* is discussed.

The animals were collected with Sherman live traps in the province of Valdivia, Chile, from July 1974 to October 1975. 11 males and 9 females were studied cytologically and classified in accordance to Osgood¹⁵. The skins and skulls were deposited in the Collection of Mammals at the Institute of Ecology and Evolution of the Universidad Austral de Chile (IEEUA). Mitotic plates were obtained by the standard air dried technique¹⁶. Chromosomes were classified according to Levan et al.¹⁷. A total of 140 good

metaphases were photographed and 75 were selected for the construction of the idiogram. The length of each chromosome is given as a percentage of female haploid set. All the specimens studied have a diploid number $2N = 56$ ($N.F. = 70$) with 21 pairs of acrocentric autosomes and 6 pairs of metacentrics (figure 1). Pair 1 is approximately one-third larger than the succeeding one. Metacentrics have values of arm ratio (r) which fluctuates between 1.45 and 1.61 (table 1, figure 2). The female karyotype exhibits a clear polymorphism in the length of the short arm of the X. 3 different forms in the analyzed females were found (figure 3a): one with 2 submetacentric X's ($r = 1.87$), 6 with one submetacentric and one subtelocentric X and 2 with both subtelocentric X's ($r = 3.65$). The differences between the values of the total length of the X's and their short arms are significant (table 2). The Y chromosome is subtelocentric. Both submetacentric and subtelocentric X's were found in males, one of which presented the former chromosomal morphology