phase-I. Linear orientation of the trivalent was also seen in some cells. Disjunction in such cells would lead to anaphase-I nuclei with 1 small plus 6 normal chromosomes and 1 small plus 7 normal chromosomes; such types of disjunction were also observed at anaphase-I.

Chromosome orientation at metaphase-I and disjunction at anaphase-I, confirm that the smaller chromosomes are telocentrics (Figures 1–3). They are unequal in size, and in karyotype they roughly correspond to the 2 arms of one of the submetacentric chromosomes. Moreover, during meiosis they do not pair with one another, but regularly pair with the 2 arms of a normal chromosome. Thus they can be considered the long arm and short arm telocentrics of a submetacentric chromosome.

The telocentrics appear to be stable in that they are observed both in root-tips and PMCs of the same plant, and when plants with telocentrics were self- or cross-pollinated, telocentrics were also present in the progeny plants.

As already noted, different types of disjunction were observed at anaphase-I leading to nuclei with 1. 7 normal chromosomes, 2. 2 telocentrics plus 6 normal chromosomes, 3. 1 telocentric plus 6 normal chromosomes and 4. 1 telocentric plus 7 normal chromosomes. Such chromosome constitutions if present in functional gametes, on appropriate combinations can produce plants in the progeny with $2n = 14$ to 16, containing 0–4 telocentrics and 12–14 normal chromosomes. One plant with $2n = 15$ (2 telos plus 13 normal) was selfed and the progeny were scored for chromosome constitutions. Among 56 plants scored, the following classes were observed; 1. 9 plants with $2n = 14$ (normal complement), 2. 40 plants with $2n = 15$ (2 telos plus 13 normal) and 3. 7 plants with $2n = 16$ (3 telos plus 13 normal).

Plants with a karyotype similar to the one described here and similar in meiotic behaviour, were also obtained from two other sources: 1. in one out of 35 plants raised from an asynaptic $X$ diploid cross and 2. in one out of 100 plants raised from $\gamma$-ray irradiated seed. Attempts are being made to find out if the telocentrics from different sources are homologous.

It would seem that these telocentrics are derived from misdivision of the centromere of a submetacentric chromosome[3,4]. In univalents, either false univalents or those resulting from asynapsis or desynapsis, the centromeres might sometimes undergo a transverse break so that each arm of the univalent becomes a new and independent chromosome with a terminal centromere. Studies of Lima-de-Faria[5,6] revealed the duplex reverse repeat chromomere pattern of the centromere, which affords a mechanical basis for centromere misdivision[3].

Telocentrics reported here in pearl millet, behave as stable chromosomes. The view that telocentrics are unstable in nature[7–9], does not seem to apply to all telocentrics. Kush and Rick[10] reported that some telocentrics in tomato were stable. More recently, origin of stable telocentrics by centric fission was reported in Chinese hamster cells in vitro[4]. Marks[3] discussed the consequences of transverse and oblique breaks at certain positions in quadripartite centromeres and pointed out how telocentrics can arise in nature. According to him, chromosomes with completely terminal contromeres can be expected as a result of centric breakage; new partial centromeres, from a transverse break, are just as efficient as the original ones, thus resulting in stable telocentric chromosomes.

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## The defensive function of cyanogenesis in natural populations[1]

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*Summary.* Detailed ecological studies of some maritime populations of Lotus corniculatus L. have shown that the distribution of the cyanogenic form of this plant is directly related to the distribution and density of molluscs which graze selectively the acyanogenic form. This work, on a genetic polymorphism, is interpreted as giving direct evidence of chemical defence in natural populations.

Most of the current reports on chemical coevolution tacitly assume that secondary plant substances have a defensive function in plants[2,3]. There is remarkably little direct evidence for this, yet a few years ago one of us[4] was able to establish that cyanogenesis in Lotus corniculatus L. did have a defensive role: the cyanogenic form of this plant was less likely to be grazed by herbivores than the acyanogenic form. This was by no means the first example of chemical defence[5–7] but it was the basis from which the criteria for establishing a defensive role for these substances were clarified[8]. It was argued, that if a plant species is monomorphic for a putative defensive substance there are no means of knowing whether a particular herbivore would eat that plant if the substance were not present. The only way out of this impasse is to study species which are either polymorphic for putative defensive substances or contain them in varying concentrations. It has been easy to demonstrate in the laboratoy selective and differential eating by

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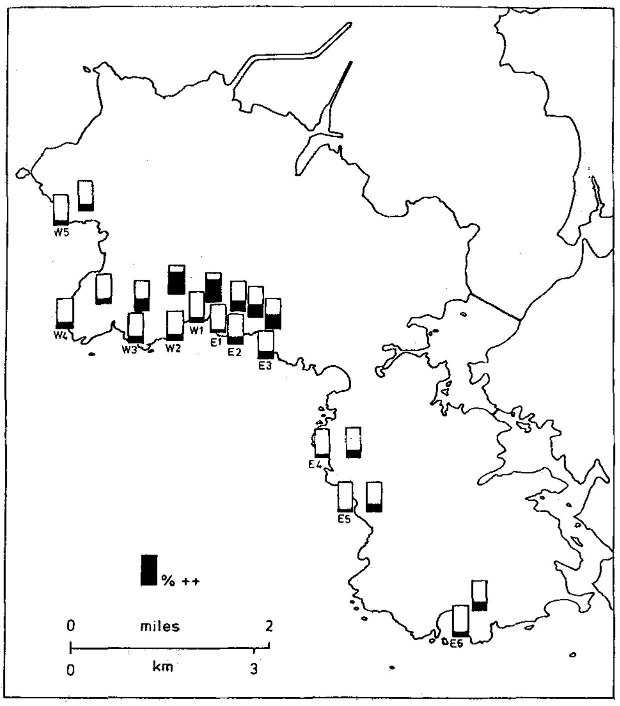
The frequency of cyanogenic plants on the coast and inland at Porthdafarch, Anglesey, from 1959 to 1975

| Year | Coast | | | Inland | | |
|---|---|---|---|---|---|---|
| | ++ | N | % | ++ | N | % |
| 1959, July | 5 | 20 | 25 | 42 | 60 | 70 |
| 1963, September | 14 | 53 | 26 | 71 | 96 | 74 |
| 1970, August | 14 | 50 | 28 | 89 | 124 | 72 |
| 1974, May | 27 | 83 | 33 | 76 | 109 | 70 |
| 1975, October | 29 | 100 | 29 | 69 | 100 | 69 |
| Means | | | 28.2 | | | 71 |

++ = number of cyanogenic plants, N = sample size, % = frequency of cyanogenesis.

herbivores of the acyanogenic form of both L. corniculatus and Trifolium repens L.[9]. On the other hand, it has been much more difficult to demonstrate this effect in natural habitats but some evidence that selective grazing of T. repens does occur outside the laboratory has been obtained[10,11]. Clearly the most suitable habitats in which to study the effects of selection are those where there are differences in the frequency of the cyanogenic form between groups of plants of the same species growing close together, for example, where a significant change in frequency occurs over a distance of metres rather than kilometres.

Ecological genetics of cyanogenesis. A suitable location was found around the bay at Porthdafarch on Holy Island, Anglesey (53° 20′ N, 4° 40′ W), where in 1959 Jones[12] found a decrease in the frequency of acyanogenic L. corniculatus, working inland from the sea cliff. Subsequent tests over the next 16 years showed that the frequency distribution of cyanogenic plants was stable (table). The habitat consists of rough pasture, short turf and scattered Calluna heath. It is subjected to minimal management with only sporadic grazing by cattle and by sheep. Neither of these species avoids grazing cyanogenic plants, because each contains an efficient detoxification system based on the enzyme rhodanase[13]. Detailed quantitative analyses of the floristic, edaphic and microclimatic variation at Porthdafarch[14] showed that the most significant environmental differences between the coast and the inland sites were related to the prevailing strong south westerly winds and to windborne salt from sea spray. Whether these factors influence the polymorphism directly or indirectly depends on the wind and salt tolerance of the different phenotypes of L. corniculatus as well as the possible effects of wind and salt in the environment of the known grazers of the plants.



The distribution of cyanogenic Lotus corniculatus along the south west coast of Holy Island, Anglesey.

If we start from the hypothesis that cyanogenesis is a protection from selective herbivores then 2 predictions about the coastal biota in Anglesey can be made and tested. Firstly, where the frequency of cyanogenic plants is high there should be some selective herbivores. Secondly, similar sites along the coast with respect to the environmental variables measured should show a similar distribution in the frequency of cyanogenic plants. That is, at sites on the cliffs close to the sea which are more exposed to wind and windborne salt, there should be a low number of selective herbivores and the frequency of cyanogenic plants should be low, whereas at sites immediately inland from these, which are less exposed to wind and windborne salt, the number of selective herbivores and the frequency of cyanogenic plants should both be higher. Of the known selective herbivores of acyanogenic plants (molluscs and small mammals) mollusc species predominate in this area. Using the quantitative techniques of catch per unit effort and of quadrat sampling (0.25 $m^2$) only a few individuals of Cochlicella acuta (Müller) were found on the exposed cliff sites. Inland, however, this species was much commoner and, what is more important, these inland sites were also the habitats of significant numbers of Helix aspersa (Müller), Cepaea nemoralis (L.), Helicella virgata (L.) and Arion ater (L.)[14]. On the cliff, for example, C. nemoralis was never found whereas inland it occurred at a frequency of 9 individuals per quadrat. Therefore the conditions of the first prediction are satisfied.

To test the second prediction, 5 sites to the west and 6 to the east of Porthdafarch were selected for study. At each site a sample of 50 L. corniculatus plants from the exposed cliff tops and a sample of 50 from inland were tested for cyanogenesis. These sites were sampled in May or July and resampled in October 1975. The mean frequencies of cyanogenic plants in the 2 tests were plotted as histograms on the map of the area (figure). These results clearly fulfil the conditions of the second prediction.

*Conclusions.* Thus we have a clear association between the occurrence of molluscs and a high frequency of cyanogenic plants and we also find that both are associated with reduced exposure to wind and with lower concentrations of windborne salt. The most reasonable explanation is that the windborne salt determines the distribution of the molluscs – it is well known that many garden gastropods can be deterred by NaCl or sel de mer – and that these

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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 work[15] 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 examined 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.

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## 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 genome[6]. Diploid strains of A. nidulans are also unstable producing sectors, which are originated from mitotic crossing-over or haploidization[7-9]. Environmental changes, as the presence of certain drugs in the culture medium, mutagenic agents or even genetic factors[10-16], 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 condition[15]. 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 drugs[17]. 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 Yellow | Green | Other* | Total |
|---|---|---|---|---|---|
| 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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