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THE KARYOTYPE OF THE LAND SNAIL *CEPAEA NEMORALIS* (L.)

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SUMMARY

A technique is described for obtaining mitotic figures from embryonic tissue. The karyotype is described and some evidence presented of a structural difference between two allopatric populations. The implications of the results are discussed.

1. INTRODUCTION

THE shell colour and banding polymorphism of *Cepaea nemoralis* (L.) has been the subject of numerous ecogenetic studies. The purpose of most of these has been to elucidate factors which may be responsible for intercolony variation in morph frequencies (see review Clarke *et al.*, 1978). A few of these studies claim to demonstrate relatively straightforward relationships between a particular physical factor and a particular allele of one or other of the relevant loci (*e.g.* Jones, 1973), but in most cases simple associations have not been found (*e.g.* Cain and Currey, 1963). Clarke (1968) has suggested that as a result of differences in genetic architecture between populations, epistatic interactions will be different in different gene pools, so that a particular selective pressure may not have the same outcome in different populations.

Differences in genetic architecture can be both absolute and relative. Absolute differences could result from point mutations. Relative differences can be brought about by structural rearrangements such as crossing-over, but also by inversions and translocations. This type of reorganisation could produce different position effects and thus promote different epistatic interactions. The general importance of changes of this kind has long been known. Lewontin and White (1960) have demonstrated that in the grasshopper *Moraba scurra*, pericentric inversions on two different chromosomes are not distributed randomly with respect to each other; the authors conclude that epistasis produces differences in genotypic fitness. At present there is no cytological evidence of intercolony differences in genotypic reorganisation in *Cepaea*, other than differences conferred by variations in crossing-over. It would clearly be of interest to determine whether other structural differences occur, and in particular to determine whether such differences can be related to otherwise inexplicable differences in the distribution of the genes for the shell polymorphism.

The chromosomes of land molluscs are small, numerous, and difficult to see. In the absence of suitable preparation techniques most cytological studies have been confined to establishing chromosome number; little attempt has been made to elucidate chromosome morphology (*e.g.* Stern, 1974).

The haploid chromosome number of *C. nemoralis* is 22 (Perrot, 1938). This figure has been confirmed by Bantock (1972) who provides a provisional classification of the chromosomes. The cytological data described by these authors have been obtained from preparations of the ovotestis. Although this organ provides large numbers of cells in meiosis, mitotic figures (which are required for detailed karyotype analysis), are few and when present are of poor morphology with the chromosomes often lacking visible centromeres (Price 1975).

In order to provide a detailed karyotypic analysis of *Cepaea* it is necessary to obtain a large number of well-defined mitotic metaphase plates. The purposes of this paper are to describe a new technique for the preparation of mitotic metaphases from embryonic tissue and to provide some preliminary evidence of a structural difference between two populations.

2. Materials and methods

(i) *Culture methods*

Adult snails were collected from a colony known to the author and maintained in plastic boxes containing natural chalk and damp filter paper. They were fed on carrot and porridge oats. At the beginning of the egg-laying season (May-July) small plastic pots containing damp soil were placed in the boxes. The pots were inspected daily for egg clutches; those containing eggs were transferred to a separate plastic box and left undisturbed for 5 days. After this period 40 eggs were removed from the clutch and washed in molluscan saline.

(ii) *Cytological methods*

Each egg capsule was split open and the embryo transferred to a watch-glass containing molluscan saline. The albumen sac, shell gland, and rudimentary shell were removed and the remaining tissue placed in a watch-glass containing 0·001 per cent aqueous colchicine. After 15 min. the contents were transferred to a 5 cm^3 glass centrifuge tube and spun at 1000 r.p.m. for 2 min. The supernatant was discarded and the remaining tissue pellet resuspended in a 3 : 1 methanol/acetic acid fixative. The fixative was changed three times after which the tissue pellet was resuspended in 2 cm^3 50 per cent acetic acid. A cell suspension was obtained by agitating the tissue/acid solution with a whirlmixer.

Heat-dried films were made on glass microscope slides using the technique described for meiotic preparations from the ovotestis (Bantock and Price, 1975*a*).

The slides were allowed to cool and then stained in 2·5 per cent Giemsa (R66. Gurr), rinsed in phosphate buffer (*p*H 6·8), and allowed to dry at room temperature. They were then soaked in michrome essence and mounted in michrome.

(iii) *Photographic and analytical methods*

The slides were scanned under phase contrast using a Ziess Photomicroscope III. Well-spread metaphase plates, with few or no overlapping chromosomes, were photographed on Ilfodata high contrast film and

photographic prints were made of the " best " mitoses. Each print was karyotyped provisionally by arranging the chromosomes in pairs using apparent similarity of size and centromere position as criteria. The arms of each chromosome were measured using dial calipers and the length of each chromosome expressed as a percentage of the total chromosome complement. Centromere position was determined by dividing the length of the short arm by that of the long arm, to give an arm ratio *r*. This value was used to classify each chromosome using the nomenclature suggested by Adhikary (1974). For *r* values of 1·00 the centromere is at the mid-point and the chromosome is type *M*. For *r* values between 0·99 to 0·61 the centromere is in the nearly median region and the chromosome type is nm. For *r* values of 0·60 to 0·23, the centromere is submedian and the chromosome type is *nsm*. For the purposes of this paper type *M* will be referred to as metacentric, and type *nsm* as submetacentric.

A large number of chromosome banding techniques have been attempted with this material. Although the procedures worked well with non-molluscan material, it was found impossible to band the *Cepaea* chromosomes. It was therefore necessary to adopt the traditional method of chromosome identification by measurement of length and the determination of centromere position, and where necessary to subject the data to statistical analysis.

Using the above criteria the chromosomes in each karyotype were then rearranged where necessary according to their relative size and centromere position. It was found that the six largest pairs were easily and unambiguously distinguishable, and needed no further analysis for their classification. The remaining 16 pairs present certain problems. Most of these chromosomes are very similar both in length and in *r* value. It is thus by no means certain that two chromosomes classified as a pair on these criteria only are in fact a pair of homologues; it follows that it is equally uncertain that a pair determined in this way corresponds to a particular pair in another karyotype. It is thus impossible at this stage to make comparisons between karyotypes which make assumptions about the correspondence of particular chromosomes within and between them.

3. Results

The diploid number of *C. nemoralis* is 44. This confirms chromosome counts from previous cytological studies. The karyotype can be divided into three groups (A to C, figs. 1 and 2); these do not correspond to those described by Bantock (1972) but represent a new classification based on a more detailed analysis of the chromosome complement. The groups are as follows:

Group A: four pairs of the largest metacentric chromosomes.

Group B: two pairs of the largest submetacentric chromosomes. The largest of these has a negative-staining region in the long arm.

Group C: the remaining chromosomes.

The chromosomes of group C are arranged as two rows. The upper row contains all those chromosomes with *r* values lying between 1·00 and 0·61, thus implying that they are metacentric. They are arranged as pairs, in decreasing size from left to right. The lower row contains submetacentric

chromosomes—those with r values between 0·60 and 0·23, arranged in the same way. It can be seen that the group C chromosomes in fig. 1 consist of eight pairs of metacentrics and eight pairs of submetacentrics.

The snails used for establishing a suitable cytological technique were taken from a population at Dunster in West Somerset. In view of the probable importance of variation in genetic architecture in *Cepaea*, it seemed worth while making a comparison with another population to determine whether differences could be found. *C. nemoralis* on the island of Steepholm was chosen; there are considerable differences between the island and mainland populations (the ecogenetics of both are described by Bantock, 1974, 1978 and Bantock and Price, 1975*b*) suggesting that their genetic backgrounds may differ. The karyotypes from each population have been prepared by the methods described above; one of those from the Steepholm population is given in fig. 2. It can be seen that in this karyotype, group C consists of nine metacentric pairs and only seven submetacentric pairs, suggesting that the populations differ by virtue of an asymmetrical pericentric inversion in one of the group C pairs of chromosomes.

Since it is currently not possible to classify individual chromosomes in group C the only comparisons between karyotypes that are possible are those which make no assumptions about the equivalence of particular chromosomes with each other. A Kruskal-Wallis one-way analysis of variance of the ranks of the 32 r values of the group C chromosomes for each of the 10 karyotypes has been carried out for each population. For the mainland $H_{(9)} = 4{\cdot}62$ $(0{\cdot}9 > P > 0{\cdot}8)$ and for Steepholm $H_{(9)} = 9{\cdot}016$ $(0{\cdot}5 > P > 0{\cdot}3)$ suggesting that there are no differences in the r values between the karyotypes within each population. The r values have been compared between populations by the Mann-Whitney test, treating the 320 values from each population as independent observations; $U = 47903{\cdot}5$, $t = 1{\cdot}41$ $(P = 0{\cdot}158)$. A similar result is obtained if the mean r values for the karyotypes are compared between populations. Although the comparison between populations is not significant, the difference between them is considerably greater than those within them. If the populations differ by virtue of only one inversion which is present at fixation, the statistical difference is likely to be swamped by the r values for the other 15 pairs of chromosomes which show no such change.

4. Discussion

The cytological technique described in this paper allows a more detailed analysis of the chromosomes of *Cepaea* than has hitherto been possible. It is clear that although most of the chromosomes are small their morphology is sufficiently clear to allow their classification. It is to be hoped that *G* and or *C*-banding procedures will eventually allow individual chromosome pairs to be identified. Although this is currently not possible, there is a suggestion from the data that the two populations differ in at least one pair of chromosomes. If such a difference can be confirmed it raises interesting questions relating to its role in promoting genetic differences between populations. Clearly it will be of interest to attempt to band the chromosomes and to examine further populations.

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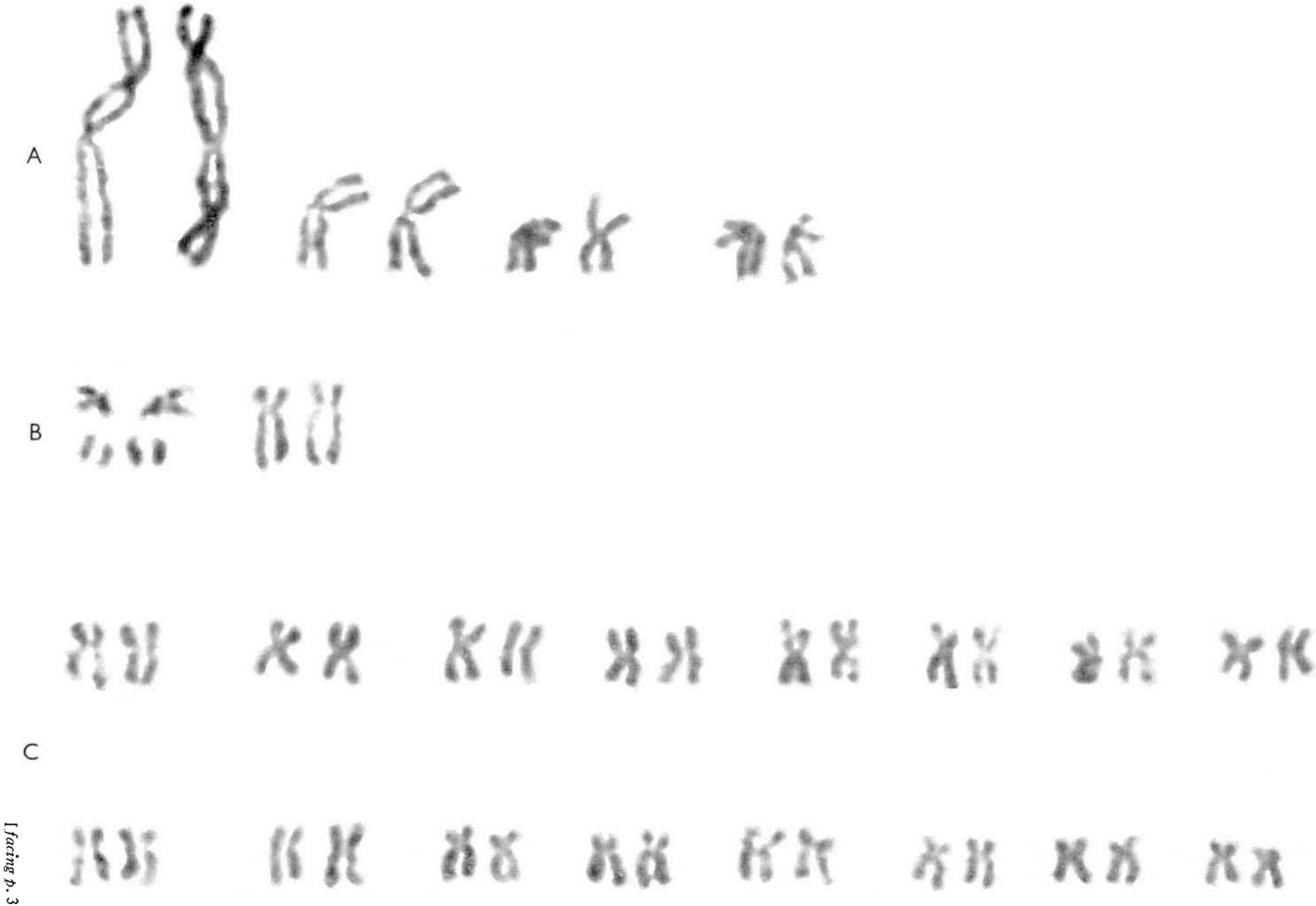

FIG. 1.—The karyotype of *Cepaea nemoralis* from Dunster, West Somerset. The chromosomes are arranged in three groups, A, B and C; the chromosomes within each group are arranged in order of decreasing size.

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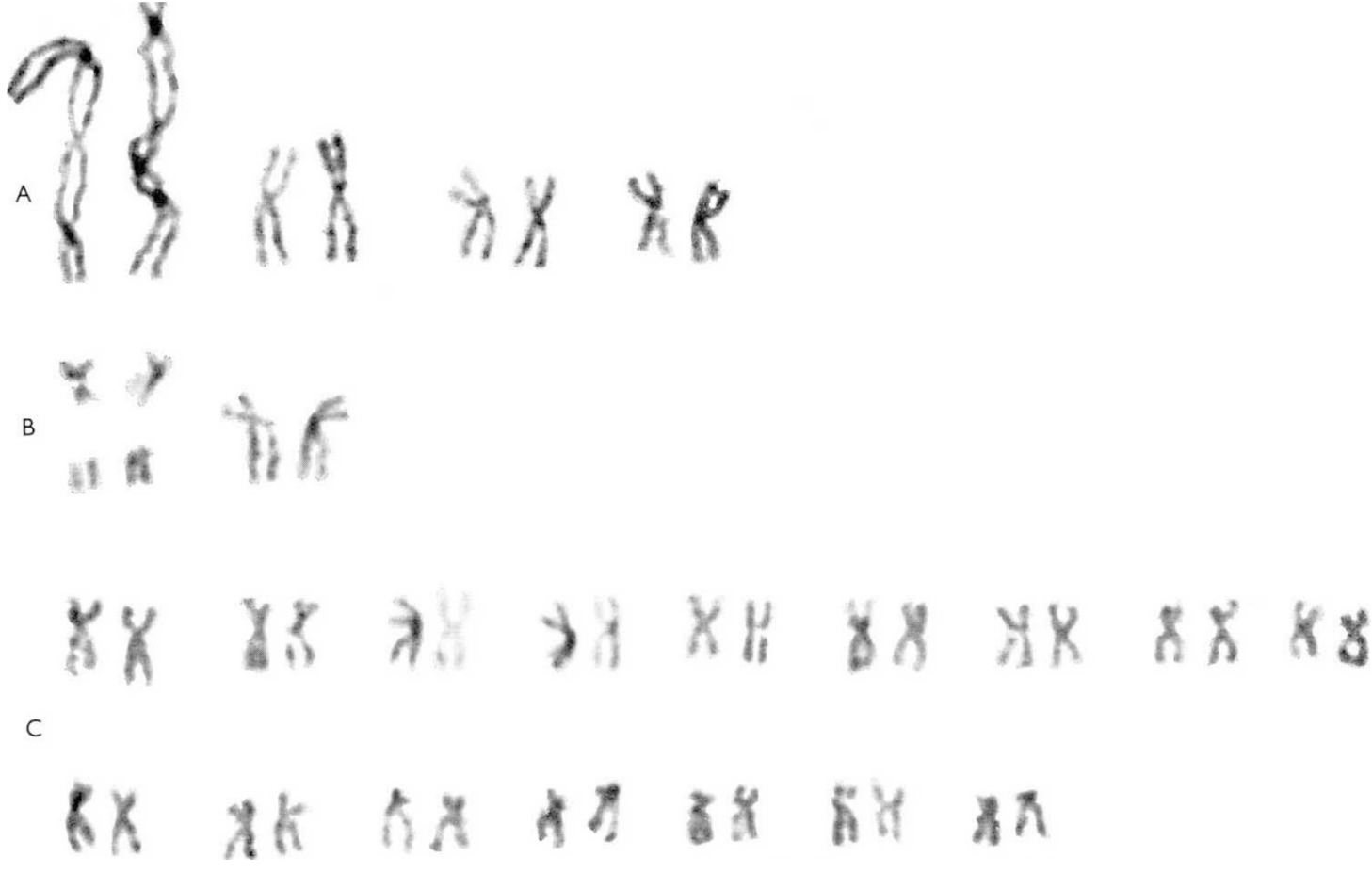

FIG. 2.—The karyotype of *Cepaea nemoralis* from the island of Steepholm. The chromosomes are arranged in three groups, A, B and C; the chromosomes within each group are arranged in order of decreasing size.

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