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17. Samples were air- and oven-dried. Examination for phenolics was carried out at the University of Strathclyde, Department of Pharmaceutical Chemistry, Phytochemical Laboratory. Nutrition samples were processed by Dr. M. J. Trlica, Department of Range Science, Colorado State University, Fort Collins.
18. In Cameroon all trees of 50 cm or more in circumference were enumerated, in a strip 5 m wide and totaling 1.45 ha (*3*). In this area, 383 individual trees belonging to 51 species were counted. In Uganda, all trees of 10 m or more in height were enumerated, in a strip 5 m wide and totaling 1.43 ha. In this sample, 469 trees, representing 51 species, were counted.
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23. Phenolic compounds might be expected to predominate among the chemical defenses of plants that occur in pure stands and in other plants or plant parts that are "apparent" to herbivores in space or time, such as long-lived leaves (*24*). Higher phenolic content of leaves of common Douala-Edea trees might be partly a consequence of longer leaf life-spans than in the Kibale site, or of a greater tendency of trees to grow in pure stands. These features may in turn be consequences of lower mineral availability (*1*). It should be noted that there is no lack of other types of secondary compounds in the vegetation of this site. Nonprotein amino acids, volatile oils, alkaloids, naphthoquinones, cyanogenic glycosides, and unusual compounds such as trimethoxystyrene have all been identified in samples from this site (*28*).
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35. For example, the coefficient of variation (*C*) of the total phenolic content of *Markhamia* mature leaves (N = 13) is only 27 percent. The 13 samples were collected from eight individuals, in five different months. For *Dichostemma* mature leaves (N = 8; each sample was taken from a different individual during the same month), C = 13 percent. For *Coula edulis* mature leaves (N = 8, collected in the same manner as *Dichostemma*), C = 30 percent.
36. We thank C. Bach, B. Hazlett, M. Huston, D. Janzen, P. Regal, and T. Swain for critical comments. D.M. and T.T.S. were supported by the Center for Field Research and Conservation, New York Zoological Society, and C.N.M. was supported by a scholarship from the British Council. J.S.G. was supported by grants RR 01055-02 and RR00167-17 from the National Institutes of Health. These studies were carried out while J.S.G., D.M., and C.N.M. were investigators of ONAREST, Institut de Recherches Agricoles et Forestières, Douala, Cameroon.

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Giemsa-11 Staining of Chromosome 1: A Newly Described Heteromorphism

Abstract. *Sequential Giemsa-11 and C-band staining of the heterochromatic region of chromosome 1 from 30 unrelated individuals revealed a high degree of variability within this region, more than was identifiable with either stain alone. The Giemsa-11 stained material usually appeared as a single band of only slightly varying size within the heterochromatic region. The position of this band ranged from a location immediately adjacent to the centromere, to one farther along the long arm or at the junction of the C-band heterochromatin and euchromatin. Two individuals had a chromosome 1 with no detectable Giemsa-11 band but an average-size C-band. Two others with a large heterochromatic segment by C-banding had two Giemsa-11 positive bands. Additional studies of five members of one family were consistent with transmission of these heteromorphisms in codominant Mendelian fashion.*

Individual morphological differences between homologous human chromosomes (heteromorphisms) occur in limited regions of specific chromosomes and have no apparent phenotypic effects. With the C-banding technique one can identify a prominent block of heterochromatin in the secondary constriction region of chromosome 1. This heterochromatin varies in size among individuals but the variations are stable and inherited (*1*).

Further variation within the C-band region (*2*, *3*) is revealed by the "lateral asymmetry" technique (*4*), which stains the sister chromatids asymmetrically—one chromatid heavily stained, the other pale. These dark and light areas vary in size, and in some individuals there are alternate light and dark bands of varying size on one chromatid; the reverse pattern on the other.

The Giemsa-11 technique (*5*, *6*) produces more specific staining of the secondary constriction region of chromosome 1 than does the C-banding technique. The area stained by Giemsa-11 is bright red against a blue background and is smaller than the region stained by the C-banding technique (*5*). By use of a modification of the Giemsa-11 technique in which only two components of Giemsa dye are used (*7*), we have discovered further stable and inherited variations of the secondary constriction region of chromosome 1.

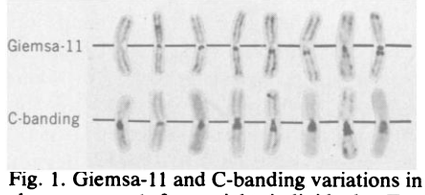

Fig. 1. Giemsa-11 and C-banding variations in chromosome 1 from eight individuals. Top row from left to right: chromosome 1 with no Giemsa-11 positive staining; five chromosomes with single bands at varying distances from the centromere; two chromosomes with two Giemsa-11 bands. Bottom row: C-banding of chromosome 1 from the same individuals as above.

Peripheral blood lymphocytes from 30 unrelated individuals were cultured for 66 hours and the chromosomes harvested by routine methods. The slides were air-dried and aged for a minimum of 2 days. They were incubated at 37°C for 2 minutes in 50 ml of fresh phosphate buffer, *p*H 11.3. Next, 0.6 ml of 1.0 percent aqueous azure B (Mc/B) and 0.5 ml of 0.25 percent eosin Y in methanol were added to the buffer and incubation was continued an additional 5 to 6 minutes. After being rinsed in distilled water, the slides were air-dried and examined with a Zeiss photomicroscope. They were considered understained if the chromosomes were uniformly pale blue and overstained if the chromosomes were uniformly pink-red. The chromosome 1 heterochromatin was stained best when both the centromeric region of chromosome 9 and the short arm–satellite regions of the acrocentric chromosome were pink-red with pale blue euchromatin. These chromosome preparations were destained and reexamined by a C-band technique (*8*).

The red-staining Giemsa-11 positive material in chromosome 1 usually appeared as a single block or band which was always within the C-band region. The amount of this material varied among the 60 different chromosomes from the 30 individuals. However, the size variation between individuals was difficult to distinguish quantitatively from the variation found from cell to cell in the same individual. The placement distinctly varied between these chromosomes but was consistent for a given chromosome as evidenced by reproducibility from cell to cell. Thirty-five of the 60 chromosomes had a Giemsa-11 positive band immediately adjacent to the centromere. Twenty-one had single Giemsa-11 positive bands at varying dis-

tances from the centromere but always within the heterochromatic region. Two chromosomes with very long C-band regions had two Giemsa-11 positive bands. This seems to support the idea that such elongated regions arise by duplication of smaller ones. Two other chromosomes had no detectable Giemsa-11 positive band, although the C-band of the same chromosome was of moderate size (Fig. 1). Some of the chromosomes with morphologically identical C-band regions had differences with Giemsa-11 banding, and some chromosomes with identical Giemsa-11 bands had different C-bands. There was no direct correlation between the Giemsa-11 and C-band variants. Thus with sequential staining the detectable variation was greatly increased.

Study of five members from one family showed inheritance of the Giemsa-11 variants and the corresponding C-band variants intact through three generations (Fig. 2). A Giemsa-11 study of 77 members of a family with the autosomal dominant disorder, antithrombin III deficiency, was undertaken because many family members were homozygous for C-banding morphology of chromosome 1 and uninformative for linkage. In this family, the Giemsa-11 and C-band variants were also inherited as a unit. Linkage information was markedly increased (*9*) because there were many instances of heterozygosity with Giemsa-11 banding in individuals homozygous with C-banding.

In order to further define the Giemsa-11 heteromorphisms, we compared Giemsa-11 and C-band variants with those produced by the lateral asymmetry technique (*2*). These studies in five unrelated individuals included one individual with a 1/15 translocation which permitted unequivocal chromosome 1 homolog identification. The blocks of heterochromatin identified with the lateral asymmetry technique corresponded in location to the Giemsa-11 positive and negative regions (Fig. 3).

It has been shown by annealing procedures in situ (*10*) that the heterochromatic region of chromosome 1 contains at least four different species of repetitive DNA (*11–13*). Since more than one species of repetitious (satellite) DNA may hybridize to a single chromosome site, Gosden *et al.* (*13*) hypothesized that the centromeric regions of such chromosomes contain adjacent blocks of different satellite DNA sequences. Giemsa-11 staining has been correlated with the presence of a specific species of repetitive DNA (satellite III) in both the human (*12, 14, 15*) and chimpanzee (*14*).

Our data suggest that the Giemsa-11 positive material is contained in discrete blocks on chromosome 1 with variable position within the heterochromatic region. The Giemsa-11 stain appears to reveal a specific subset of heterochromatin. The C-banding technique is less selective and stains the entire heterochromatic region known to contain highly repetitive DNA. These findings together with annealing studies in situ support the idea that the heterochromatic region is composed of blocks of different satellite DNA sequences (*13*).

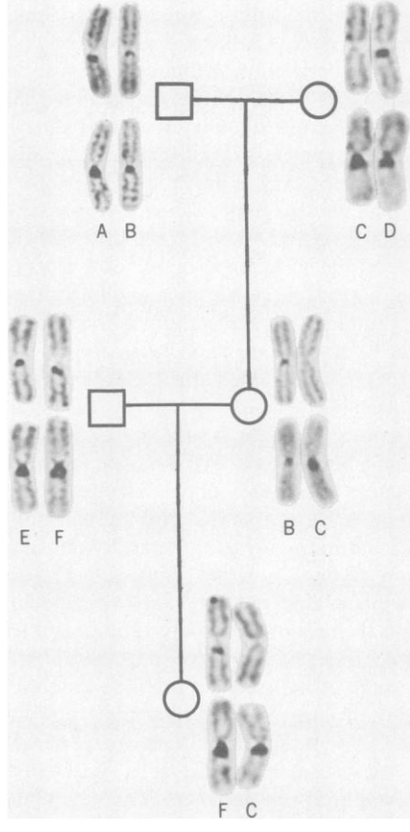

Fig. 2. Inheritance of sequentially stained chromosomes 1 in three generations of a family. Note three chromosomes labeled *C* without a Giemsa-11 positive band but with a moderate size C-band.

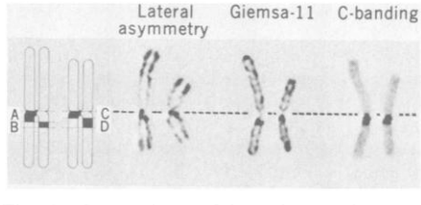

Fig. 3. Comparison of homologous heterochromatic regions of chromosome 1 with three staining techniques. Chromosomes from an individual with a 1/15 translocation were used to permit homolog identification; the longer chromosome with the translocation is on the left. The ideogram depicts the regions stained with the lateral asymmetry technique. Blocks *B* and *C* stain with Giemsa-11, whereas blocks *A* and *D* do not. With C-banding all four blocks are stained.

Of special interest is the finding that these blocks can vary both in location and amount and can be duplicated or deleted without apparent phenotypic effects. In addition, these Giemsa-11 heteromorphisms may be of value not only in gene mapping and linkage studies but also in the elucidation of the organization and structure of heterochromatic regions.

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