Phenolic Content of Vegetation in Two African Rain Forests: Ecological Implications

Abstract. *Mature leaves of abundant trees in rain-forest vegetation on acid white-sand soils of the Douala-Edea Reserve, Cameroon, contain approximately twice the concentration of phenolic compounds found in similar rain-forest vegetation on lateritic soils of the Kibale Forest, Uganda. Phenolics are the most widespread class of ecologically important plant secondary compounds. This study provides support for the hypothesis that vegetation on low-nutrient soils contains relatively high concentrations of chemicals deterrent to herbivores and pathogens. Unlike the two species of* Colobus *monkeys found in Kibale Forest, black colobus monkeys (*Colobus satanas*) in the Douala-Edea Reserve avoid the leaves of almost all abundant tree species in the area, selectively feeding on leaves of relatively rare components of the vegetation (deciduous trees and second-growth vines). Unlike any other colobine studied to date, black colobus in the Douala-Edea Reserve feed heavily on seeds, which contain lower concentrations of phenolic compounds than do leaves in this site.*

The cost of herbivory to a plant should be reflected in the magnitude of the plant's investment in chemical defenses. Janzen (*1*) reasoned that the cost of replacing materials eaten by herbivores would be greater in areas of nutrient-poor soils than for plants growing on sites richer in nutrients. He predicted that vegetation growing on impoverished white-sand soils would be found to contain greater concentrations of herbivore-deterrent toxic secondary compounds (such as tannins, saponins, and alkaloids) than would vegetation growing on more nutrient-rich soils. Information for evaluating this prediction has been scant. We present data from a long-term comparative community study of chemical defenses in trees of two African rain forests on strikingly different soils. These data, gathered as part of our studies on the ecology of anthropoid primates (*2–4*), permit evaluation of this hypothesis.

Our studies were carried out in the Kibale Forest, Uganda, and the Douala-Edea Reserve, Cameroon. Both sites support evergreen forest. Struhsaker (*4*) has described the vegetation of the Uganda site [which is adjacent to the study area of Oates *et al.* (*5*)], and Letouzey (*6*) has presented the results of a reconnaissance of the Cameroon site. The forest there is characterized by the abundance of *Lophira alata* (Ochnaceae), the most common large emergent, and by dominance of the families Euphorbiaceae, Caesalpiniaceae, Guttiferae, Olacaceae, and Ebenaceae. The Kanyawara study area in Kibale Forest shows no family dominance, aside from a preponderance of Ulmaceae (*4*). Soils of the Kanyawara study area of the North Kibale Forest, Uganda (*4*), are dark gray to red sandy loams or sandy clays underlaid by strongly folded and metamorphosed sedimentary rocks (*7*). Fertility assessment of these soils ranges from "fair" through "favorable" to "good" (*7*). Soils of the Douala-Edea Reserve (*8*) on the coast of Cameroon contrast with those of the Kibale study site both in derivation and composition. These extremely sandy soils have developed on beach sand deposited by a longshore current of the Atlantic Ocean. Because deposition has continued (at varying rates) since the end of the Cretaceous era, the sandy sediments are extensive and deep (*9*). Douala-Edea soils contain small amounts of silt and clay and average about 86 percent sand; surface soil may comprise 100 percent sand. Differences between the two sites in total nitrogen, phosphorus, and potassium are not large in view of the differences in soil structure and ion-retention properties, but there is a major difference in soil acidity. While *p*H of the Kibale soils varies little about the mean, *p*H 5.92, the surface *p*H of the Douala-Edea site is much lower, averaging 3.92 ($P < .005$, Mann-Whitney U test); figures as low as *p*H 2.7 were obtained (*10*). At this degree of acidity, nitrifying bacteria are inhibited, and minerals such as phosphorus and molybdenum become largely unavailable (*11*). The relative poverty of the soil of this site is reflected in the low rates of regeneration after clearing and the poor performance of farms and gardens on these soils. Significantly lower concentrations of nitrogen, phosphorus, and ash in the vegetation of this site (Table 1) also suggest lower nitrogen and mineral availability. Also suggestive of such a difference is the sparsity of deciduous trees in the Douala-Edea site (*12*).

We expect that the per-unit cost of replacing minerals and other resources lost to herbivores (including photosynthate, whose accumulation is dependent on minerals) would be greater for plants in the Douala-Edea site than at Kibale. If Janzen's hypothesis is correct, plants of the Douala-Edea site should contain relatively high concentrations of toxic secondary compounds. Circumstantial evidence that this may be the case has been presented (*3*), but up to now no systematically gathered information has been available.

Phenolics are the most widespread major class of ecologically important secondary compounds. This group includes simple phenolic acids, flavonoids, polyphenols such as tannins, other classes of compounds common in plants, and many compounds of more restricted distribution. Their general toxicity, bacteriostatic and fungistatic properties (*13–16*), and the absence of any general role for most phenolics in the internal economy of plants (*14*) support the view that the phenolics owe their wide distribution and their often high concentrations to se-

Table 1. Chemical composition and energetic value of mature leaves of common trees of Kibale and Douala-Edea study sites.

Assays	Kibale*		Douala-Edea		Mann-Whitney test (*34*)	
	Mean	Range	Mean	Range	U	*P*
Percent ash†	12.1	5.48–26.2	3.7	1.5–5.7	193	<.001
Percent N	2.8	1.9–4.3	1.7	1.3–2.5	189	<.001
Percent P	0.171	0.096–0.286	0.086	0.035–0.141	176.5	<.001
Total phenolics (Folin Denis)‡	35.2	12.3–107.8	75.7	13.2–153.1	181.5	<.005
Vanillin-HCl§	6.6	0–95.1	30.4	0.9–90.5	178	<.005
Proanthocyanidin¶	25.5	0–166.3	46.4	4.1–270.3	178.5	<.005
Gross energy (cal/g)	4204.3	3198–4751	4833.9	4223–5253	178	<.001
Percent digestibility#	29.5	6.44–57.8	13.7	3.3–45.6	152	<.01
Gross energy × percent digestibility	1215.3	288.5–2416.4	653.1	170.3–2184.1	148	<.025

*In both sites, for phenolics assays $N = 16$ at Cameroon and 14 at Uganda and for nutrient assays $N = 14$ at both sites. †Three species of Ulmaceae in the Kibale Forest are silica accumulators. Mean value for percent ash of Kibale leaves excluding these three species is 9.5, which is still significantly higher than the Douala-Edea values ($P < .001$). ‡Milligrams per gram as tannic acid. §Milligrams per gram as catechin/epicatechin. ¶Milligrams per gram as quebracho tannin. #Results for the digestibility assay are the proportion of dry matter digested after 96 hours by rumen inoculum from a fistulated cow fed 80 percent corn and 20 percent prairie hay.

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lective advantages conferred by their deterrence of herbivores and pathogens.

We collected mature leaves of many of the most common tree species in each site and examined them for content of phenolic compounds and their nutrient content (*17*). Strip enumeration of trees in each study site permitted us to identify the most abundant tree species (Table 2). Young leaves were also collected when available. Our comparisons of phenolic content of mature leaves deal with 16 of the 20 most abundant species in the Cameroon site, and 14 of the 20 most abundant species in the Uganda site. These species comprise 79.4 percent (Cameroon) and 83.3 percent (Uganda) of the total number of trees in the size classes enumerated (*18*). We are thus able to characterize a major proportion of the canopy mature-leaf vegetation of each site.

We adopted three assay procedures to obtain information on quantity and type of phenolic compounds present. In each case, a standard, the mature leaf of *Sorindeia mildbraedii* (Anacardiaceae), was tested with each batch of assays. The total quantity of phenolics was assessed by the Folin-Denis reagent in a modification of the AOAC assay (*19*). While not giving a perfectly stoichiometric assay, this reagent is relatively unaffected by the degree of polymerization of the phenolics and probably provides the best available assessment of total phenolic content. Attempts to measure more specific types of phenolic moiety were made by (i) the vanillin-HCl assay which reacts with compounds possessing an undeactivated ring system based on resorcinol (*20*) and (ii) the proanthocyanidin assay for condensed tannins (*21*).

Table 1 compares the results of these three assays of phenolic compounds in mature leaves of abundant trees in the two sites. For all three assays, mean values for the Cameroon samples are significantly greater than those for the Uganda samples. Mean concentrations of total phenolics in the Cameroon leaves is twice that of the Uganda leaves; values for the vanillin and proanthocyanidin assays that measure condensed tannins and their monomers are both at least two times higher in the Cameroon leaves. Drying and storage of plant material (our samples were air- and oven-dried) can result in qualitative and quantitative changes in phenolic compounds (*22*). However, samples from each site were treated similarly, and, while the values obtained may underestimate the absolute values, it is unlikely that artifacts introduced by drying can account for the consistent differences between the two sites. Relative differences, rather than absolute values, are pertinent to our hypothesis.

Table 2. Means of phenolics by the Folin Denis (FD), vanillin-HCl (V), and proanthocyanidin (PA) assays of mature leaves of abundant trees, Douala-Edea and Kibale sites. The means are expressed as described in Table 1.

Tree species	Percent of individuals enumerated	Samples (No.)	Mean value*		
			FD	V	PA
	Douala-Edea				
Protomegabaria stapfiana	42.0	2	43.12†	38.70	37.74
Sorindeia mildbraedii	5.0	1	44.00	8.20	51.00
Anthonotha macrophylla	3.7	4	77.88	20.75	43.86
Garcinia mannii	3.4	1	153.12	4.67	7.14
Uapaca staudtii	3.1	1	68.20	90.45	96.90
Coula edulis	2.9	8	89.76	13.12	24.48
Leptaulus sp.	2.9	3	13.20	0.90	4.08
Lophira alata	2.6	1	64.24	36.90	44.37
Diospyros bipindensis	2.4	1	112.64	22.96	39.27
Dichostemma glaucescens	2.1	8	110.0	79.05	70.89
Librevillea klainei	1.8	1	27.28	22.71	59.67
Berlinia auriculata	1.8	1	48.84	16.24	33.15
Pachypodanthium cf. *confine*	1.8	1	84.48	62.32	128.52
Mammea africana	1.6	1	150.92	56.66	270.30
Strombosia pustulata	1.3	2	35.20	3.28	85.68
Maprounea membranacea	1.0	4	85.80	9.18	12.24
	Kibale				
Diospyros abyssinicus	20.0	1	45.76	1.48	5.61
Markhamia platycalyx	17.7	13	20.68	0.08	1.02
Celtis durandii	10.4	1	12.32	0.00	0.00
Uvariopsis congensis	7.7	1	19.80	0.08	2.55
Teclea nobilis	6.4	1	48.84	0.00	0.00
Funtumia latifolia	4.5	2	45.32	9.51	23.97
Strombosia scheffleri	4.5	1	18.92	0.00	13.26
Parinari excelsa	3.2	1	107.80	34.19	36.72
Chaetacme aristata	2.6	1	12.32	0.00	0.00
Millettia dura	2.4	2	40.48	4.02	24.48
Dombeya mukole	1.3	1	17.16	53.96	59.16
Cassipourea ruwenzoriensis	1.1	1	53.68	95.12	166.26
Bosqueia phoberos	0.9	1	25.96	0.82	10.71
Celtis africana	0.6	1	16.72	0.00	15.30

*Though sample sizes for each species are small in most cases, there seems to be little intraspecific variation in phenolic content in those species for which numerous samples are available (*35*). Remaining among the 20 most abundant tree species in the Uganda forest (and their percentage representation in the enumeration) are *Pancovia* cf. *turbinata* (1.9), *Neoboutonia macrocalyx* (1.1), *Linociera johnsonni* (0.9), *Lovoa swinnertonii* (0.9), *Olea welwitschii* (0.9), and *Aphania senegalensis* (0.6). †The unexceptional phenolic content of this very abundant species leads us to suspect that it may contain other types of secondary compounds, since both its young and mature leaves are completely avoided by black colobus. Tree species among the 20 most abundant that remain to be examined in the Cameroon site (and their percentage representation in the enumeration) are *Anthocleista* sp (2.1), *Pausinystatia* sp. (1.8), undentified Rosaceae (1.6), and unidentified Flocourtiaceae (1.0).

For the 16 tree species abundant in the Douala-Edea study area (Table 2), the mean content of phenolics, as estimated by our assays, is roughly equivalent to 7.6 percent (dry weight), and the mean content of condensed tannins is about 4.6 percent (Table 1). In two of these species, *Garcinia mannii* and *Mammea africana* (both Guttiferae), concentration of phenolics in the leaves exceeds 15 percent (dry weight). Plants growing in dense stands on exceptionally poor soils within the reserve often contain concentrations of phenolics higher than the average for this site, as predicted (*1*). We have obtained information on phenolic content of some of these, which, although not among the 20 most abundant species in the main study area, are locally important components of the vegetation. Mature leaves of *Strephonema pseudocola* (Combretaceae), which forms virtually pure stands in some swamps, contain approximately 16 percent phenolics. Species of *Garcinia* (Guttiferae) are often locally dominant in the Douala-Edea forest; mature leaves of those so far examined contain high concentrations of phenolics. In contrast, the mean concentration of phenolics in mature leaves of trees from the Kibale site (Table 2) is 3.5 percent, and the mean content of condensed tannin is 2.6 percent. The highest value for total phenolics is 10.8 percent (*Parinari excelsa*, Rosaceae, a common large emergent) (*23, 24*).

Numerous experimental studies have documented the effects of various phenolic compounds on herbivores (*13–15*). Small amounts of tannin in the diet can

have growth-inhibitory effects (*15*). Tannins have the capacity to form cross-linked complexes with proteins that are stable to conditions encountered in the gut and resistant to proteolysis (*25*). Ingestion of tannins results in greatly increased excretion of nitrogen in the feces, leading to malnutrition or even death (*15*). Tannins and other phenolics may also be oxidized by polyphenoloxidases, produced by either the plant or the herbivore, to quinones, which react covalently with various functional groups of proteins and free amino acids (*26*). Formation of *o*-quinones from *o*-dihydroxyphenols may lead to lowered quality of plants as forage for domestic animals (*27*). Many phenolics other than tannins are also toxic (*13*). A variety of uncommon phenolics have been identified in samples from the Douala-Edea site. These include insecticidal *n*-propylcoumarins (*Mammea africana*) as well as xanthones (several species of Guttiferae), biflavonoids (*Garcinia mannii*), naphthoquinones (*Diospyros* spp.), and other compounds about whose toxicity little is known (*28*).

We believe that the high content of tannins and other phenolic compounds in vegetation of the Douala-Edea site has major implications for the ecology of animals dwelling there. For example, red colobus (*Colobus badius*) and black-and-white colobus monkeys (*C. guereza*) in the Kibale Forest are, like most colobines, predominantly leaf eaters, each species obtaining at least 75 percent of its diet from leaves or leaf parts and utilizing the leaves of a number of common trees. *Colobus guereza*, for example, feeds heavily on leaves of *Celtis durandii*, an abundant tree in which the contents of tannins and other phenolics are low (*5*). In contrast, black colobus monkeys (*C. satanas*) in the Douala-Edea Reserve obtain only 37 percent of their diet from leaves (*29*). When feeding on leaves, this monkey (i) avoids the mature leaves of all and the young leaves of most of the common tree species and (ii) feeds selectively on leaves of relatively rare deciduous trees and of herbaceous second-growth vines, leaves of which have been postulated to contain lower concentrations of secondary compounds (*1, 30*). Unlike any other colobine so far studied, the black colobus of the Douala-Edea Reserve feeds considerably on seeds, which constitute 53 percent of their annual diet. Probably as a consequence of its avoidance of the leaves of common trees and its restriction to relatively rare and seasonal items, *C. satanas* exists at a density roughly one-tenth that of the combined density of the two *Colobus* species in the Kibale Forest. Densities of other anthropoid primates in the Douala-Edea site are also very low in comparison to other African rain-forest sites (*2–4, 31*), as predicted by Janzen's hypothesis (*1*).

Tannins are inhibitory to growth because they lower the herbivore's digestive efficiency (*15*). With the use of rumen inoculum from a fistulated cow, assays of dry matter digestibility were performed on samples from the two study sites. Of the digestibility assays in common use, this one was thought to be most applicable to colobus monkeys, whose ruminant-like digestion presents striking convergences with that of true ruminants (*32*). Digestibility was negatively correlated with the phenolic content ($N = 60$; Spearman rank correlation coefficient $r_s = -.5011$, $P < .001$) and with the content of condensed tannin (proanthocyanidin assay) ($N = 60$; $r_s = -.4915$, $P < .001$). Although the gross energy content of mature leaves of common Douala-Edea trees is significantly higher than that of Kibale trees, their digestibility is significantly lower. Approximate "net energetic value" (gross energy multiplied by the proportion of dry matter digested) of the Kibale leaves is almost twice that of the Douala-Edea leaves (Table 1). We propose that, whereas *Colobus* in the Kibale study area can obtain phenolic-poor leaves from some common tree species (*5*), higher concentrations of phenolic compounds render most Douala-Edea leaves unsuitable as food for *Colobus*. We cannot, however, discount the idea that avoidance of leaves by *C. satanas* in the Douala-Edea Reserve is due simply to the significantly lower contents of nitrogen and minerals in leaves from this site (Table 1) or to possible differences in content of lignin and cellulose, about which we have no information.

Full evaluation of the significance of avoidance of leaves by *C. satanas* awaits further information on the phenolic content of young leaves, which constitute the bulk of the diet of both Ugandan *Colobus* species. Even at currently available sample sizes, however ($N = 7$ at Cameroon, $N = 10$ at Uganda, with only the 20 most abundant tree species in each site being considered), young leaves from the Uganda site are significantly lower ($U = 56$, $P = .025$) in total phenolics than those from Cameroon, and the difference (in the same direction) in content of condensed tannins approaches significance ($U = 52$, $P \cong .05$).

Our study provides empirical support for the hypothesis that vegetation on soils of low nutrient-supplying capacity contains high concentrations of secondary compounds. The existence of blackwater rivers in the Cameroon site and the low population densities of anthropoid primates are features consistent with the hypothesis (*1*). We suspect that vegetation of many white-sand areas would present even stronger contrasts to the phenolic-poor vegetation of Kibale Forest, since nutrient supply to the Douala-Edea vegetation may be increased by nutrient input from the nearby ocean (*33*).

Our data suggest that high content of phenolic compounds in leaves in the Cameroon site may explain the general avoidance of leaves and great selectivity in leaf feeding shown by *Colobus satanas*. It is interesting that seeds, the major item class utilized by this monkey, contain consistently lower amounts of phenolic compounds than do leaves in this site (*28*).

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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.

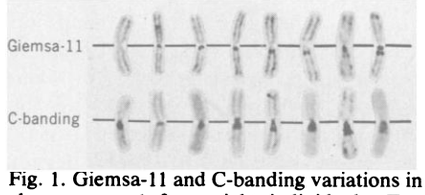

Fig. 1. Giemsa-11 and C-banding variations in chromosome 1 from eight individuals. Top row from left to right: chromosome 1 with no Giemsa-11 positive staining; five chromosomes with single bands at varying distances from the centromere; two chromosomes with two Giemsa-11 bands. Bottom row: C-banding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

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