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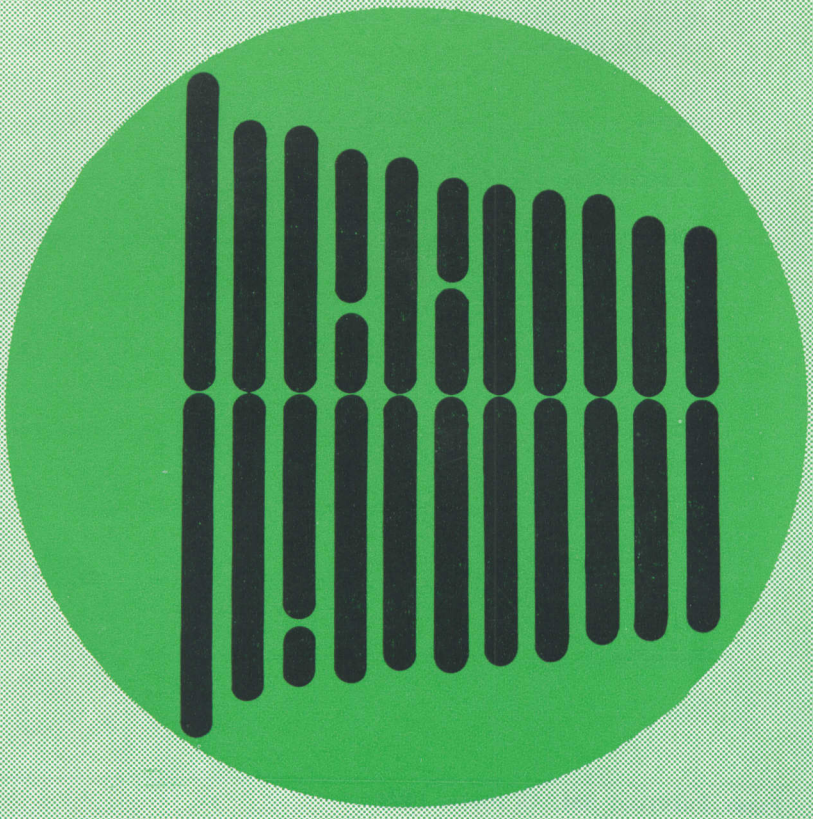
A Karyotypic Study of Cypresses Indigenous to the Southwestern United States



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A Karyotypic Study of Cypresses Indigenous to the Southwestern United States

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INTRODUCTION

In 1964, Auburn University initiated a program of genetic tree improvement among several species of cypress indigenous to the southwestern United States. Its purpose was to develop varieties of cypress suitable for Christmas tree production in the Southeast. Part of this program has involved accumulation of basic cytogenetic information necessary to determine the inherent karyotypic variation within the particular group of species under study.

Cypresses native to the United States have not been thoroughly examined karyotypically, and there are several possible reasons. First, the genus *Cupressus* has not been important commercially. Secondly, the difficulty of seed collection because of widely scattered and isolated natural stands, coupled with low seed viability and resultant poor germination, have undoubtedly been deterrents to investigation. A third reason might be the presence of numerous, long chromosomes, characteristic in general of all conifers, which renders separation, measurement, and identification of individual chromosomes extremely difficult. Finally, the problem of morphological differentiation of species has also precluded more extensive study.

This study was undertaken in order to help clarify the karyotypes of *Cupressus* and to further delineate the natural variation

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which occurs within the genus. It presents findings which corroborate the generally accepted haploid number of 11 for *Cupressus* and describes several variants which show the presence of one or more accessory chromosomes, heretofore rarely observed in woody plants. A total of seven species of *Cupressus*, including four varieties of *C. arizonica* and two varieties of *C. goveniana*, are represented.

REVIEW OF LITERATURE

Sax and Sax *(13)* first reported a basic number of n = 11 for the Cupressaceae. Their findings on the genera *Thuja, Juniperus,* and *Chamaecyparis* indicated that the chromosomes of all three were similar in morphology and more or less isobrachial (metacentric). Several studies since then have corroborated the basic number of n = 11 for the Cupressaceae and shed additional light on the karyotypes of *Cupressus.* In 1936, Numata and Yamashita (cited by Kanezawa *(5)*) reported 2n = 22 for *Cupressus lusitanica* var. *benthamii.* Camara and DeJesus *(2)* observed meiosis in *C. lusitanica* and reported a number of irregularities which included formation of univalents, multivalents, translocation rings, anaphase bridges, and fragments. They reported a basic number of n = 11 chromosomes. Mehra and Khoshoo *(8)* found 11 chromosomes in gametophytic tissue of *C. funebris* and *C. torulosa* and 22 chromosomes in root tips of *C. sempervirens.* For the first two species, they reported a karyotype of 1 heterobrachial chromosome and 10 approximately isobrachial chromosomes. In addition, a satellite, which was "somewhat thicker" in *C. funebris,* was found in one of the median-submedian chromosomes of both species. For the diploid set of *C. sempervirens,* Mehra and Khoshoo observed two heterobrachial chromosomes, each of which carried a secondary constriction in its long arm. Finally, they observed meiosis in pollen mother cells of *C. cashmeriana, C. lusitanica,* and *C. arizonica* and reported a normal meiosis with formation of 11 bivalents in each of these species.

The most extensive study of the karyotypes of *Cupressus* was reported by Hunzicker *(4)* who worked with root tips. He reported karyotypes for seven species which included, *C. arizonica, C. funebris, C. glabra, C. lusitanica, C. macrocarpa, C. sempervirens,* and *C. torulosa.* All of these showed a basic number of 2n = 22 except for *C. glabra,* which had an extra, small accessory chromosome that gave it a number of 2n = 23. In addition, all species had median-submedian centromeres with the exceptions

of *C. lusitanica* var. *lusitanica* and *C. torulosa,* each of which had two heterobrachial chromosomes. Finally, he observed three types of secondary constrictions. The first of these divided one of the chromosome arms so that a linear satellite was formed (distal section of the arm longer than the proximal) and the second so that a rounded satellite was formed (distal section shorter than the proximal section). The third type was described by Hunziker as an "anucleolar secondary constriction," rarely seen, and possibly an artifact due to variations in the cell's environment. All of the species showed the first type of constriction while only *C. lusitanica* var. *lusitanica* and *C. lusitanica* var. *benthamii* had the second type.

MATERIALS AND METHODS

Seeds used in the present study were collected in the southwestern and western United States during the summer of 1964 and included the following species and varieties:

Population and species	Location
1. *C. arizonica* Greene var. *arizonica*	Big Bend National Park, Texas
2. *C. arizonica* Greene var. *arizonica*	Chihuahua, Mexico
3. *C. arizonica* Greene var. *arizonica*	Chiricahua National Monument, Arizona
4. *C. arizonica* Greene var. *arizonica*	Greenlee County, Arizona
5. *C. arizonica* Greene var. *arizonica*	Cochise Stronghold, Arizona
6. *C. arizonica* Greene var. *arizonica*	Portal, Arizona, Cave Creek, Cochise County
7. *C. arizonica* Greene var. *arizonica*	Pima County, Arizona, Bear Canyon
8. *C. arizonica* var. *glabra* Sudw., Little	Oak Creek Canyon, Arizona
9. *C. arizonica* var. *glabra* Sudw., Little	Gila County, Arizona
10. *C. guadalupensis* S. Wats.	Guatay Mountain, San Diego County, California
11. *C. arizonica* var. *stephensonii* Wolf, Little	Cuyamaca Peak, San Diego County, California
12. *C. sargentii* Jepson, Little	Cypress Creek, Monterey, California
13. *C. macrocarpa* Hartw.	Monterey County, California
14. *C. goveniana* Gord. var. *goveniana*	Santa Cruz County, California
15. *C. goveniana* Gord. var. *pygmaea* Lemmon	Mendocino County, California
16. *C. bakeri* Jepson	Siskiyou County, California
17. *C. bakeri* Jepson	Shasta County, California
18. *C. macnabiana* A. Murr.	Amador County, California
19. *C. arizonica* var. *nevadensis* Abrams, Little	Kern County, California
20. *C. arizonica* Greene var. *arizonica*	Graham County, Arizona

Each of the above populations has been fully described by Posey and Goggans *(10).* In the identification of species, the taxonomic treatment given by Little *(6)* has been used.

PRETREATMENT AND STAINING SCHEDULE

Only somatic tissue obtained from root tip meristems was used in karyotype determination. It was originally intended to examine karyotypes from at least two seedlings from each of three parent trees from every 1 of the 20 populations, thus giving a minimum of six observations per population with an overall total of 120 observations. However, in a few instances poor germination coupled with inability to obtain a plate suitable for measurement resulted in fewer observations than the minimum of six per population. In other instances it was possible to obtain more than the minimum number, so the final results were based on a total of 172 measurements. Seeds from each of the populations were germinated either in petri dishes filled with moistened vermiculite and placed in a growth chamber or in trays filled with a sand/soil mixture and placed in a greenhouse.

Root tips from newly germinated seedlings were excised when they reached about 5 mm. in length and placed in a solution of 8-hydroxyquinoline (0.3 g./liter) for 24 hours at 10°C to contract the chromosomes and to arrest cell division at metaphase *(11)*. They were then fixed in Farmer's fixative (3 parts absolute alcohol: 1 part glacial acetic acid) for 24 hours, hydrolyzed in either 1N HCL at 60°C or in 1 part absolute alcohol: 1 part concentrated HCL at room temperature for 10 minutes, and stained in Schiff's reagent (Feulgen) for 1-2 hours. After each root tip had been stained, it was put into 45 per cent acetic acid for 10 minutes, transferred to a microscope slide where it was immersed in a drop of acetocarmine, and squashed.

CHROMOSOME MEASUREMENT AND DETERMINATION OF KARYOTYPE

Microscopic slides were examined for cells which had mitotic chromosomes well spread out and, as nearly as possible, in one plane. At 1,125X magnification, such cells were examined and photographed using 35 mm. black and white film. The negatives were then mounted in slide holders and projected onto white paper so that the largest chromosomes measured approximately 6 centimeters. Each enlarged image was closely compared with the original cell viewed through the microscope so that each chromosome on the image could be outlined to show such details as the position of the centromeres, location of secondary constrictions, and location of ends that might be hidden through overlapping.

Chromosomes of a given cell were then arbitrarily numbered from 1-22, and individual arms were measured from their ends. Centromere regions were not included in the measurements, and when a chromosome arm was curved it was measured along a series of straight lines tangent to the arc described by the curve. The longer of the two arms of a given chromosome was designated the "a" arm; the shorter, the "b" arm *(11)*. Presence and position of secondary constrictions were recorded whenever they occurred, and these were designated types 1, 2, and 3 in accordance with the descriptions offered by Hunzicker *(4)*. Types 1 and 2 corresponded respectively to the linear and rounded constrictions found by him, while type 3 fitted his description of a diffuse anucleolar constriction, rarely seen and possibly an artifact.

After all of the chromosomes of a cell had been measured, individual lengths were converted to relative values to permit comparisons between cells. This was done by computing average chromosome length for the cell and relating absolute lengths to this average *(16)*:

$$\text{Relative length} = \frac{\text{Absolute length}}{\text{Average length}} \times 100$$

Chromosomes were then arranged in descending order of total relative length, and homologous pairs were chosen by careful reexamination of the original microscope slide and matching of similar arm lengths. In determining pairs the shorter arm was considered to be of greater diagnostic value since it was less susceptible to stretching *(11)*. Once paired, the lengths of matched arms were averaged in order to arrive at the haploid karyotype of each cell.

Population mean relative lengths of each chromosome in the karyotype were calculated. The standard errors at the 5 per cent level were calculated for the population means of the longest and shortest chromosomes.

RESULTS

Chromosome Numbers

Most karyotypes examined from each population possessed a diploid complement of 22 chromosomes with median to submedian centromeres (short/long arm ratio 0.5-1.0) which confirms results of previous cypress chromosome investigations. However, there were five populations, 4, 8, 10, 19, and 20, that exhibited some tendency toward abnormality in chromosome numbers. Within

each of these, individuals were found which possessed an extra chromosome (2n = 23), and in addition, in the samples from populations 8 and 20, one individual in each had 2n = 24 chromosomes.

The aneuploid individual in the sample from population 10 possessed an extra chromosome which did not differ greatly in total relative length from the smallest member of the diploid set and could possibly be a trisomic. Table 1 presents the haploid karyotype of this abnormal seedling. The extra chromosome was identified through the pairing of similar arm lengths and could not be recognized by microscopic observation since it had no features to readily distinguish it from other chromosomes of similar length.

TABLE 1. HAPLOID KARYOTYPE OF AN ANEUPLOID INDIVIDUAL FROM POPULATION 10, PARENT 1. (2n = 23)

Chromosome	Total relative length	Relative length*	
		a	b
1	130	70	60
2	112	61^2	51
3	109	59	50^1
4	107	58	49
5	105	57	48
6	97	52	45
7	95	57	38
8	94	52	42
9	92	51	41
10	87	51	36
11	81	49	32
12**	74	39	35

* Secondary constrictions of types 1 and 2 are indicated by small numbers adjacent to the arms in which they occur.

** Extra chromosome that is possibly the result of a simple duplication of an entire chromosome (a trisomic).

In all other cases of abnormality in chromosome numbers the extra individual(s) could be identified at a glance. They were approximately half the size of the smallest chromosome in the diploid set and had median to submedian centromeres. Figures 1 and 2 show these extra chromosomes while Appendix Table 1 gives the karyotypes of all seedlings that possessed them and Table 2 presents the average karyotype of these seedlings. Chromosomes observed in several cells were in the process of duplication, and in these instances the accessory chromosome(s) replicated in a regular fashion and apparently had fully functional centromeres. Figure 3 illustrates this by showing normal replication of an accessory chromosome. The remaining chromosome complement of these abnormal cells did not differ greatly from that of individuals with

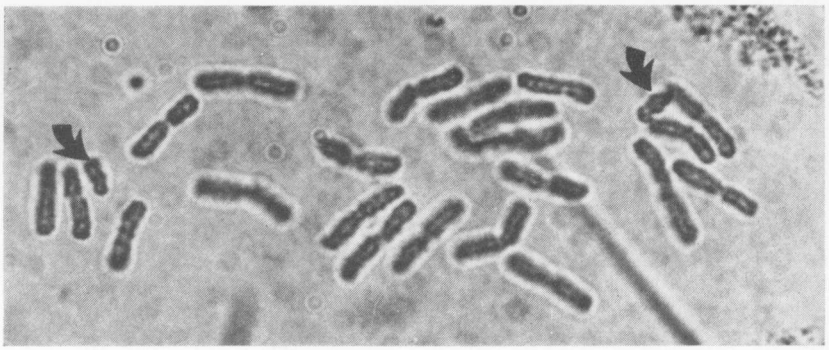

FIG. 1. Chromosomes of Cupressus arizonica var. arizonica from a seedling of population 20, parent 10; (2n = 24). Arrows point to the extra chromosomes.

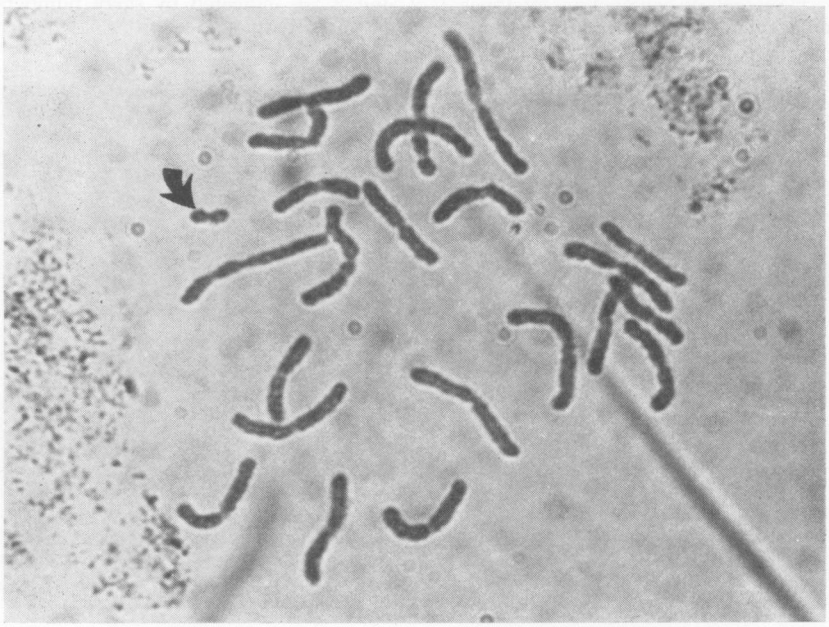

FIG. 2. Chromosomes of Cupressus arizonica var. nevadensis from seedling of population 19, parent 4; (2n = 23). Arrow points to the extra chromosome.

diploid numbers of 22 except for a tendency of the two longest members to have a larger relative length than normal. For two parent trees, no seedlings were found to have normal chromosome numbers. These were parent 2, population 19 and parent 5, population 20. However, it may be that further examination would reveal some individuals with 2n = 22 since only two of the seedlings obtained from each parent were examined.

TABLE 2. AVERAGE HAPLOID KARYOTYPE OF INDIVIDUALS WITH EXTRA CHROMOSOMES.* (2n = 23 and 2n = 24)

Chromosome	Total relative length	Relative length*	
		a	b
1	133	70	63
2	119	62	57
3	113	60[2]	53
4	110	58	52[1]
5	106	58	48
6	102	55	47
7	99	54	45
8	95	52	43[3]
9	90	51	39
10	87	50	37
11	83	48	35
12**	36	21	15

* Secondary constrictions of types 1, 2, and 3 are indicated by small numbers adjacent to the arms in which they appeared most frequently.

** Small accessory chromosome(s).

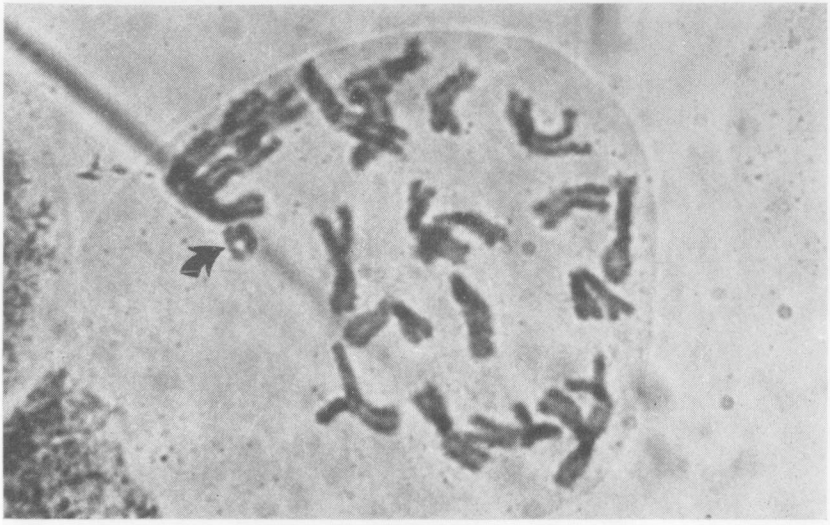

FIG. 3. Photomicrograph that shows a supernumerary chromosome of a seedling from population 19, parent 4 which has duplicated in a regular manner. Arrow points to the extra chromosome.

Chromosome Length

Karyotype differences between parents within a population or between populations cannot be accurately ascertained on the basis of relative chromosome lengths unless the differences are quite large and there are peculiar features about the chromosomes which can be used for identification purposes. In this study of

cypress, neither one of these criteria was met except for one or two chromosomes in each set which carried a secondary constriction which can be used as a distinguishing marker. Differential contraction, small differences in pretreatment period, bending of chromosome arms, and inability in given instances to obtain plates with the chromosomes all in one focal plane further complicate the situation. However, from the data gained in the present study it was possible to obtain a reasonably reliable estimate of the basic karyotype of each population based on the average relative lengths of the chromosomes from the various progenies. Haploid karyotypes of each population and standard errors of the population means of the longest and shortest chromosomes are presented in Appendix Table 2.

No important differences in relative chromosome lengths between populations could be detected. Karyotypes were all very similar, therefore they were combined and the average karyotype for the *Cupressus* populations studied is presented in Table 3. In examining 152 cells from seedlings which had karyotypes of 2n = 22, eight cells had longest chromosomes that were abnormal. In each case they were approximately 15 units of relative length greater than the average lengths of the longest chromosomes of the populations to which the seedlings belonged. These instances were thought to be a result of differential contraction or stretching of the chromosomes in question and not to be reflections of differences in karyotype between parents or between populations. This conclusion seems valid since measurements of additional cells from the same seedlings revealed karyotypes more near the average.

TABLE 3. AVERAGE HAPLOID KARYOTYPE OF *Cupressus* POPULATIONS STUDIED.*

Chromosome	Total relative length	Short/long arm ratio
1	130	.91
2	116	.90
3	110	.88
4	106	.86
5	102	.83
6	98	.82
7	95	.81
8	92	.82
9	88	.80
10	84	.76
11	79	.76

* Refer to Appendix Table No. 2 for relative lengths of arms and locations of constrictions.

Position of Centromeres

All chromosomes of cypress examined had their centromeres in median to submedian positions as shown by the short/long arm ratios in Table 3. Differences in the position of the centromere between numerically equal chromosomes of the various populations were not large and probably reflected errors in measurement rather than actual morphological variation.

Secondary Constrictions

Three types of secondary constrictions were identified, two of which occurred frequently enough to be taken as regular features of the karyotypes in which they occurred. The first type of constriction (type 1) occurred in the progeny of each of the various parents, regardless of population, and was most probably a nucleolar organizer region in function. It appeared as a distinct narrow band located approximately 16 relative units from the centromere, Figure 4, or occasionally as a long, tenuous strand (possibly a result of stretching during preparation of the tissue) which terminated in a linear satellite that was approximately 30 relative units in length. Most of the time the constriction appeared to be located in the short arm of the chromosome. Because of the phenomenon of chromosome reversals as described by Simak *(14)*, in which chromosomes of nearly equal length can be misoriented in a karyotype description because of differential contraction and bending, it was not possible to accurately assign the constriction to any one chromosome of the haploid set. Similarly, because of reversals of order in chromosome arms of nearly equal length, it occasionally occurred in the long arm. In Figure 5 and Appendix Table 2 the type 1 constriction is shown in the karyotype position where it occurred most frequently.

The second type of constriction (type 2) had the appearance of being the opposite of the first type and could be the result of an inversion if the possibility of misorientation because of arm reversals is considered. It was generally a distinct narrow band located approximately 30 relative units from the centromere. It occurred about half of the time in the long arm and the other half of the time in the short arm. Distal to the constriction, a small, rounded satellite nearly 11 relative units in length was formed, Figure 6. The position of type 2 in the idiogram of Figure 5 and in Appendix Table 2 was handled in the same manner as for type 1. Type 2 did not occur as frequently as did type 1 and was not

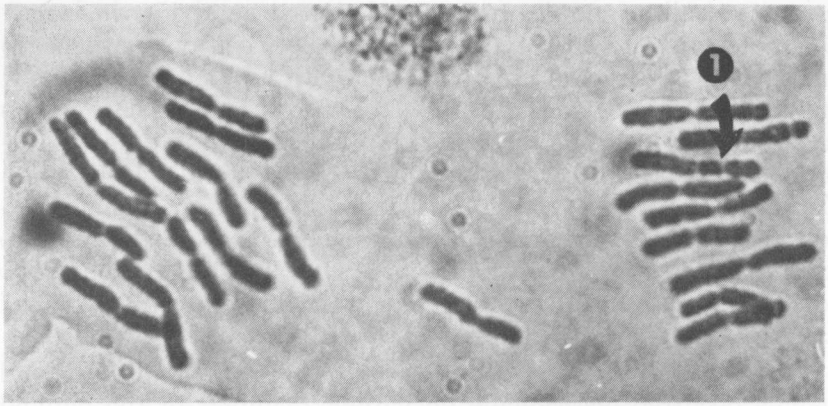

FIG. 4. Photomicrograph that shows type 1 constriction as a distinct narrow band.

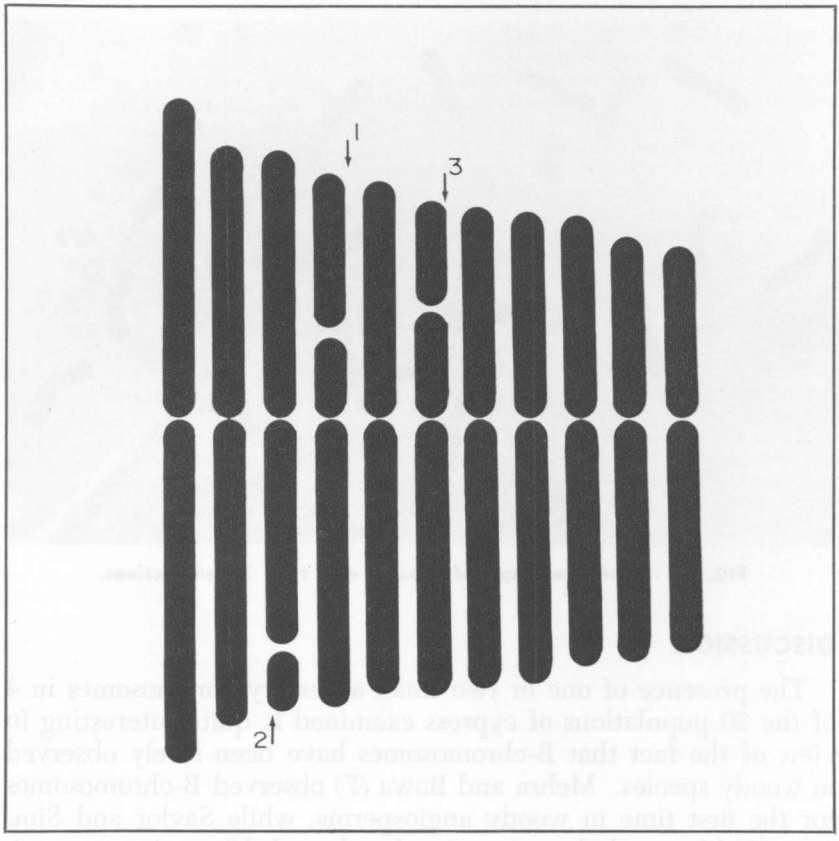

FIG. 5. Haploid idiogram of population 1.

present at all in samples from populations 13 and 15 which may be indicative of a karyotypic difference between populations. In most cases it occurred in conjunction with type 1, and relatively few differences between seedlings of the various parents within a population could be discerned on the basis of the types of secondary constrictions which they carried. In samples from populations 14 and 19, type 2 constrictions were found almost exclusively in the longest chromosome, which is less susceptible to identification errors because of its greater length. In these two cases, the position of the type 2 constrictions possibly reflected actual population differences.

The third type of constriction (type 3) was a diffuse band which occurred occasionally on various members of the haploid set (usually chromosomes 7 or 8) and may have been an artifact caused by pretreatment contraction, Figure 6.

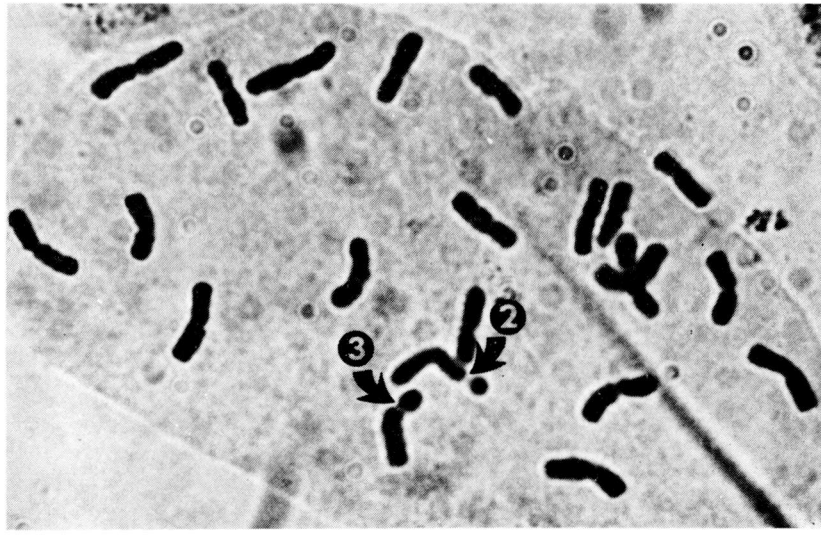

FIG. 6. Photomicrograph of type 2 and type 3 constrictions.

DISCUSSION

The presence of one or two small accessory chromosomes in 4 of the 20 populations of cypress examined is quite interesting in view of the fact that B-chromosomes have been rarely observed in woody species. Mehra and Bawa *(7)* observed B-chromosomes for the first time in woody angiosperms, while Saylor and Simons *(12)* observed them in coastal redwood (*Sequoia sempervi*

rens), a hexaploid species. The only other case of their appearance in conifers that is known to these authors is in *Cupressus glabra* as reported by Hunzicker *(4)*. Thus the present study confirmed Hunzicker's findings and indicated that B-chromosomes may occur in cypress more extensively and in larger numbers than previously reported.

The accessory individuals reported in this paper were classified as B-chromosomes even though meiosis had not been observed because they fit the generally accepted definition of supernumeraries very well.

Darlington *(3)* describes supernumerary chromosomes as having certain unique properties. They are generally small, vary in numbers among different individuals, occur in both odd and even numbers, tend toward neutrality in that they exhibit no apparent effect on the external morphology of the plant, and do not pair with members of the normal complement, or A-set, at meiosis. Furthermore, they are variable in regard to heterochromatin content and range from being completely devoid of it to being nearly completely heterochromatic.

Our observations indicated that B-chromosomes of cypress may be largely devoid of heterochromatin since examination of interphase nuclei of individuals with extra chromosomes revealed no heteropycnosis. It remains to be seen whether their presence in any way alters the phenotypic expression of the plant or affects its fertility.

Various seedlings of known female parents from population 20 were planted in 1966 and 1967 as part of progeny studies. There appeared to be wide variation within the population sample in a number of traits, including form, color, and branch angle. Whether or not this variation was correlated with the presence of B-chromosomes is not known. That supernumeraries are not necessarily genetically inert has been documented by a number of studies, and Darlington *(3)* states that "— they produce small, less specific effects and are the active basis of quantitative variation."

The matter of origin of supernumeraries in cypress is one for speculation until further studies, especially of meiosis, can be conducted. It is known, however, that B-chromosomes may be derived from the normal chromosome complement of a plant in at least three ways *(1,3,* and *15)*. First, they may arise through irregular meiotic pairing involving translocations and non-disjunction. Secondly, they could originate by fragmentation across the

centric region. The third possibility would involve crossing-over in inversion-heterozygotes coupled with inclusion of a centric fragment in a germ line which already has normal chromosomes of the same kind.

According to Darlington *(3)*, the second mechanism, that of fragmentation across the centric region, would be recognizable by examination of root tip mitoses wherein varying numbers of supernumeraries would be seen. This phenomenon would be observed since fragmented centromeres are usually incapable of regular movement. In cypress, no evidence of varying numbers of B-chromosomes between root tip cells was found, so it is unlikely that the extra chromosomes originated in this fashion. Also, all of the B-chromosomes observed in this study had median-submedian centromeres which were fully functional, an impossibility if their origin was by centric fragmentation.

The first and third possible origins then remain as more likely candidates, and there is some direct and indirect evidence that one or both phenomena may be functioning in some species of cypress. The direct evidence is that presented by Camara and DeJesus *(2)* for *C. lusitanica* in which they reported irregularities in meiosis that included formation of univalents, multivalents, translocation rings, anaphase bridges, and fragments. The indirect evidence concerns the findings of the present authors with regard to the two kinds of secondary constrictions, types 1 and 2. Measurements of relative length of the chromosome arms having these constrictions indicated that one could be the inversion of the other. In a number of instances, only one member of each type could be found in a given cell. For pairing purposes it was assumed that the constriction of the missing member merely could not be seen and that the two types of constrictions were located on non-homologous chromosomes. There were a few cells which seemed to uphold this contention by showing two chromosomes with one type of constriction and one with the other, Figure 7. Out of 172 cells examined, only 4 or 5 expressed this condition, but the possibility of an inversion involving a satellited chromosome should not be discarded without further investigation. Furthermore, samples from two populations, 13 and 15, showed no sign of the type 2 constriction, and in all seedlings of these samples, both homologues of the type 1 constriction were clearly visible.

Taking into account the limitations imposed by technique on a study such as this, the measurements of relative length revealed

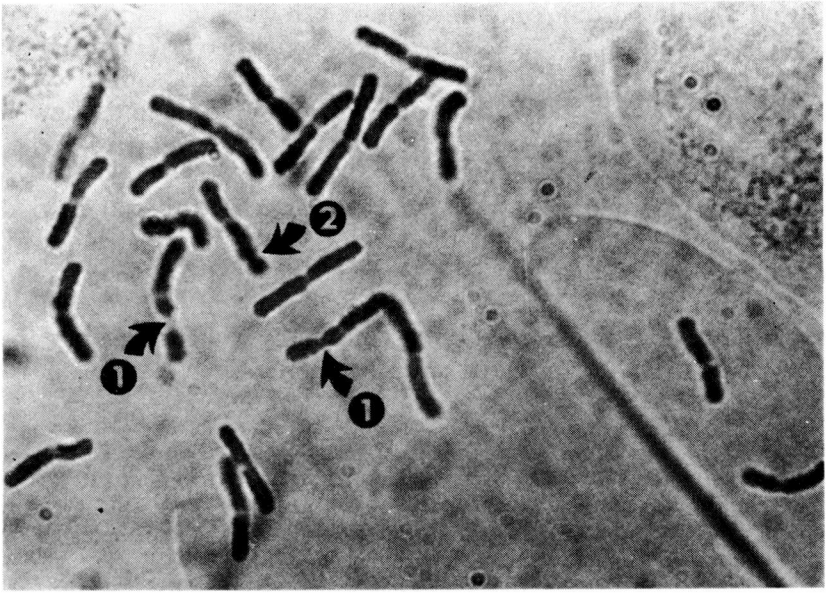

FIG. 7. Photomicrograph which shows that type 1 and type 2 constrictions apparently occur on non-homologous chromosomes.

no outstanding differences in karyotype between the various parents and populations examined. This does not mean, however, that there were no differences; without doubt, evolution has occurred at the genic level, and breeding tests may indicate specific barriers. But in gross chromosome structure, karyotypes of all populations are basically identical. Perhaps a technique such as that employed by Pederick *(9)* on female gametophyte tissue might reveal more of the ultrastructure of the chromosomes and would permit construction of a detailed chromosome map. In four samples, differences in type of constriction present or in the location of a type of constriction seemed indicative of actual population differences. Examination of seedlings from 13 and 15 revealed the absence of type 2 constrictions while those from 14 and 19 showed their presence almost exclusively in the longest chromosome. Selective breeding tests and studies of meiotic pairing behavior should greatly enhance present knowledge of the karyotypes of cypress.

SUMMARY

Karyotypes of seven species of *Cupressus*, including four varieties of *C. arizonica* and two varieties of *C. goveniana*, were pre-

sented. No important differences in relative chromosome lengths could be detected between any of the species or varieties, and all chromosomes examined had median-submedian centromeres. Nearly all species and varieties of cypress investigated showed the presence of both a linear and a rounded satellite in two apparently non-homologous members of the haploid set. However, *C. macrocarpa* and *C. goveniana* var. *pygmaea* differed in that the presence of the rounded satellite could not be detected. Finally, certain individuals of *C. arizonica* var. *arizonica, C. arizonica* var. *glabra, C. arizonica* var. *nevadensis,* and *C. guadalupensis* had one or two accessory chromosomes which in all but the case of *C. guadalupensis* appeared to be B-chromosomes.

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APPENDIX

APPENDIX TABLE 1. HAPLOID KARYOTYPES OF INDIVIDUALS WITH EXTRA CHROMOSOMES*

Population 4, Parent 11—based on two observations. (2n = 23)				Population 8, Parent 15—based on one observation. (2n = 23)			
Chromosome	Total relative length	Relative length		Chromosome	Total relative length	Relative length	
		a	b			a	b
1	127	66	61	1	141	74	67
2	120	62	58	2	113	57	56
3	113	59[2]	54	3	110	58	53
4	111	61	50[1]	4	108	56	51[1]
5	107	57	50	5	105	63	42
6	100	57	43	6	101	59	42
7	96	52	44	7	100	55	45
8	94	51	43	8	94	47	47
9	91	52	39	9	90	49	41
10	84	49	35	10	90	50	40
11	83	47	36	11	83	49	35
12**	43	28	15	12	28	15	13

Population 19, Parent 4—based on four observations. (2n = 23)				Population 19, Parent 2—based on four observations. (2n = 23)			
Chromosome	Total relative length	Relative length		Chromosome	Total relative length	Relative length	
		a	b			a	b
1	129	68	61	1	130	67	63
2	117	61	56	2	120	64[2]	56
3	114	62	52	3	113	59	54[1]
4	108	56	52[1]	4	111	57	54
5	105	59	46	5	104	57	47
6	102	53	49	6	101	54	47
7	99	53	46	7	99	57	42
8	96	56	40[3]	8	95	51	44[3]
9	92	51	41	9	91	50	41
10	89	50	39	10	87	50	37
11	82	47	35	11	84	47	37
12	36	19	17	12	32	17	15

(Cont.)

APPENDIX TABLE 1. *(Cont.)*

Population 20, Parent 5—based on four observations. (2n = 23)

Chromosome	Total relative length	Relative length	
		a	b
1	139	73	66
2	116	61	55[1]
3	112	61	51
4	109	56	53
5	105	56	49
6	102	53	49
7	97	50	47
8	94	54	40
9	88	49	39
10	87	49	38
11	82	49	33
12	37	24	13

Population 8, Parent 15—based on one observation. (2n = 24)

Chromosome	Total relative length	Relative length	
		a	b
1	133	68	65
2	128	64	64[1]
3	114	60	54
4	112	59	53
5	110	62	48
6	103	59	44
7	101	60	41
8	97	50	47
9	91	52	39
10	87	51	36
11	86	47	39
12	34	18	16

Population 20, Parent 8—based on one observation. (2n = 24)

Chromosome	Total relative length	Relative length	
		a	b
1	140	74	66
2	133	70	73
3	119	63	56
4	114	64	50[1]
5	110	57	53
6	100	59	41
7	99	52	47[3]
8	96	50	46
9	90	51	39
10	86	57	29[3]
11	77	41	36
12	38	24	14

Population 20, Parent 10—based on one observation. (2n = 24)

Chromosome	Total relative length	Relative length	
		a	b
1	140	72	68
2	117	61	56
3	113	59	54[1]
4	112	59	53
5	108	57	51
6	105	61	44
7	103	55	48
8	97	53	44
9	89	55	34
10	87	48	39
11	87	52	35
12	41	24	17

* Secondary constrictions of types 1, 2, and 3, are indicated by superior numbers adjacent to the arms in which they occur.

** Small accessory chromosome(s).

APPENDIX TABLE 2. HAPLOID KARYOTYPES OF POPULATIONS 1-20*

Population 1

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	131	68	63	.93
2	114	60	54	.90
3	110	57[2]	53	.93
4	105	57	48[1]	.84
5	101	54	47	.87
6	98	55	43[3]	.78
7	95	53	42	.79
8	94	53	41	.77
9	89	49	40	.82
10	84	48	36	.75
11	80	46	34	.74

$s_{\bar{x}}$ longest** = 1.4410
$s_{\bar{x}}$ shortest = 0.7894

Population 2

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	126	65	61	.94
2	114	60	54	.90
3	110	57	57[1]	1.00
4	105	57	48	.84
5	102	57[2]	45	.79
6	99	54	45	.83
7	96	53	43	.83
8	93	52	41	.79
9	90	50	40	.80
10	85	48	37	.77
11	81	47	34	.72

$s_{\bar{x}}$ longest = 0.8498
$s_{\bar{x}}$ shortest = 0.8461

Population 3

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	132	70	62	.89
2	115	61	54	.89
3	109	57	52[2]	.91
4	107	57	50	.88
5	102	56	46[1]	.82
6	99	56	43	.77
7	95	51	44	.86
8	93	52	41[3]	.79
9	89	53	36	.69
10	83	47	36	.77
11	77	44	33	.75

$s_{\bar{x}}$ longest = 4.0797
$s_{\bar{x}}$ shortest = 3.5862

Population 4

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	133	70	63	.90
2	116	61	55	.90
3	110	59	51[1]	.86
4	105	55[2]	50	.91
5	100	55	45	.82
6	97	52	45	.87
7	95	54	41	.76
8	93	50	43	.86
9	89	50	39	.78
10	85	49	36	.73
11	76	43	33	.77

$s_{\bar{x}}$ longest = 1.8864
$s_{\bar{x}}$ shortest = 2.2047

Population 5

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	132	69	63	.91
2	116	60	56	.93
3	111	59	52[2]	.88
4	106	57	49[1]	.86
5	101	54	47	.87
6	98	54	44	.81
7	95	53	42	.79
8	92	50	42	.84
9	89	49	40[3]	.82
10	84	44	35	.71
11	78	44	34	.77

$s_{\bar{x}}$ longest = 1.1546
$s_{\bar{x}}$ shortest = 0.9043

Population 6

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	131	72	59	.82
2	118	59	59	1.00
3	106	60	46	.77
4	104	58	46	.79
5	101	54	47[1]	.87
6	98	52	46	.88
7	98	53	45	.85
8	92	49	43	.88
9	88	50	38	.76
10	86	50	36	.72
11	82	45	37	.82

$s_{\bar{x}}$ longest = 0.3500
$s_{\bar{x}}$ shortest = 2.5000

(Cont.)

APPENDIX TABLE 2. *(Cont.)*

Population 7

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	130	69	61	.88
2	118	62	56	.90
3	110	58	52	.90
4	105	56	49[2]	.88
5	102	54	48[1]	.89
6	98	57	41	.72
7	94	51	43[3]	.84
8	92	51	41	.80
9	88	49	39	.80
10	84	48	36	.75
11	80	47	33	.70

$s_{\bar{x}}$ longest = 2.9711
$s_{\bar{x}}$ shortest = 1.5275

Population 8

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	127	67	60	.90
2	115	61	54	.89
3	111	59	52[2]	.88
4	105	56	49[1]	.88
5	101	55	46	.84
6	98	55	43	.78
7	96	53	43	.81
8	92	53	39[3]	.74
9	89	50	39	.78
10	87	47	40	.85
11	80	46	34	.74

$s_{\bar{x}}$ longest = 2.0403
$s_{\bar{x}}$ shortest = 0.7046

Population 9

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	129	69	60	.87
2	116	62	54	.87
3	110	61	49[2]	.80
4	107	57	50[1]	.88
5	102	56	46	.82
6	99	54	45	.83
7	95	52	43	.83
8	92	49	43[3]	.88
9	88	46	42	.91
10	86	52	34	.65
11	78	44	34	.77

$s_{\bar{x}}$ longest = 4.0987
$s_{\bar{x}}$ shortest = 2.5905

Population 10

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	127	67	60	.90
2	116	63	53	.84
3	110	61	49[2]	.80
4	106	56	50[1]	.89
5	103	56	47	.84
6	98	55	43	.78
7	96	54	42	.78
8	92	51	41	.80
9	88	50	38	.76
10	85	48	37	.77
11	82	48	34	.70

$s_{\bar{x}}$ longest = 2.0428
$s_{\bar{x}}$ shortest = 0.8818

Population 11

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	132	67	65	.97
2	119	63	56	.89
3	108	58	50[2]	.86
4	105	56	49	.88
5	101	55	46[1]	.84
6	99	53	46	.87
7	94	53	41	.77
8	93	51	42[3]	.82
9	85	49	36	.73
10	84	46	38	.83
11	80	44	36	.82

$s_{\bar{x}}$ longest = 4.2064
$s_{\bar{x}}$ shortest = 1.8393

Population 12

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	130	68	62	.91
2	117	63	54	.86
3	109	59	50[2]	.85
4	106	58	48	.83
5	103	56	47[1]	.84
6	99	54	45	.83
7	96	53	43[3]	.81
8	92	52	40	.77
9	90	49	41	.84
10	83	47	36	.77
11	75	44	31	.70

$s_{\bar{x}}$ longest = 1.3627
$s_{\bar{x}}$ shortest = 1.7573

(Cont.)

APPENDIX TABLE 2. *(Cont.)*

Population 13

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	132	68	64	.94
2	113	61	52	.85
3	111	58	53	.91
4	105	56	49[1]	.88
5	102	55	47	.85
6	98	56	42	.75
7	95	51	44	.86
8	93	51	42[3]	.82
9	88	49	39	.80
10	85	49	36	.73
11	80	44	36	.82

$s_{\bar{x}}$ longest = 1.7872
$s_{\bar{x}}$ shortest = 0.7222

Population 14

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	128	68	60[2]	.88
2	116	61	55	.90
3	109	59	50	.85
4	105	57	48[1]	.84
5	102	57	45	.79
6	99	55	44	.80
7	96	53	43	.81
8	92	52	40	.80
9	88	48	40	.83
10	85	49	36	.73
11	80	45	35	.78

$s_{\bar{x}}$ longest = 1.8846
$s_{\bar{x}}$ shortest = 0.8459

Population 15

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	129	67	62	.93
2	116	63	53	.84
3	109	58	51	.88
4	106	56	50	.89
5	102	56	46[1]	.82
6	98	55	43	.78
7	96	54	42	.78
8	92	50	42[3]	.84
9	88	50	38	.76
10	84	49	35	.71
11	79	46	33	.72

$s_{\bar{x}}$ longest = 1.2384
$s_{\bar{x}}$ shortest = 1.3079

Population 16

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	130	69	61	.88
2	117	61	56	.92
3	110	58	52[2]	.90
4	108	59	49	.83
5	103	56	47[1]	.84
6	98	54	44	.81
7	96	51	45	.88
8	93	50	43	.86
9	90	49	41	.84
10	84	47	37	.79
11	80	44	36	.82

$s_{\bar{x}}$ longest = 2.4757
$s_{\bar{x}}$ shortest = 0.5947

Population 17

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	133	69	64	.93
2	116	61	55	.90
3	108	57	51	.89
4	105	57	48[1]	.84
5	101	54	47	.87
6	97	54	47	.87
7	95	52	43	.83
8	91	49	42	.86
9	90	52	38	.73
10	84	47	37	.79
11	80	44	36	.82

$s_{\bar{x}}$ longest = 1.6225
$s_{\bar{x}}$ shortest = 0.9128

Population 18

Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	126	66	60	.91
2	116	62	54	.87
3	110	57	53	.93
4	106	57	49	.86
5	101	56	45[1]	.80
6	98	52	46	.88
7	96	53	43	.81
8	93	52	41	.79
9	89	49	40	.82
10	84	47	37	.79
11	81	47	34[3]	.72

$s_{\bar{x}}$ longest = 1.2292
$s_{\bar{x}}$ shortest = 1.2292

(Cont.)

APPENDIX TABLE 2. *(Cont.)*

Population 19					Population 20				
Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio	Chromosome	Total relative length	Relative length a	Relative length b	Short/long arm ratio
1	128	67[2]	61	.91	1	128	67	61	.91
2	114	60	54	.90	2	120	61	59	.97
3	109	57	52	.91	3	110	57	53[2]	.93
4	104	55	49[1]	.89	4	109	59	50[1]	.85
5	102	55	47	.85	5	102	58	44	.76
6	99	53	46	.87	6	101	54	47	.87
7	96	54	42	.78	7	94	55	39	.71
8	94	50	44[3]	.88	8	91	50	41	.82
9	90	51	39	.76	9	86	48	38	.79
10	85	48	37	.77	10	82	46	36	.78
11	81	45	36	.80	11	78	44	34	.77
	$s_{\bar{x}}$ longest = 1.1758					$s_{\bar{x}}$ longest = 3.8441			
	$s_{\bar{x}}$ shortest = 0.9573					$s_{\bar{x}}$ shortest = 1.1546			

* Secondary constrictions of types 1, 2, and 3 are indicated by small numbers adjacent to the arms in which they occur.

** $s_{\bar{x}}$ = standard deviation of the mean.