

## Patterns of heterochromatin distribution in plant chromosomes

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### Abstract

The C-band distribution patterns of 105 angiosperm species were compared to identify general patterns or preferential sites for heterochromatin. The base-specific fluorochrome reaction of heterochromatin for 58 of these species and the role played by the average chromosome size in band distribution were also considered. The results showed that heterochromatin was preferentially located in similar chromosome regions, regardless of the distance from the centromere. This trend results in generalized bands, with heterochromatin distribution being identical in most chromosomes of a karyotype. Such bands very often displayed the same fluorochrome reaction, suggesting possible repeat transfer between non-homologous sites. Chromosome size may also play a role in heterochromatin location, since proximal bands were much more common in small-sized chromosomes.

### INTRODUCTION

Heterochromatin (HC) is one of the chromosomal components that has attracted most attention from cytogeneticists, because of its still unknown function, its apparent lack of genes and the fact that it stains differently to the rest of the chromosome. Especially after the development of HC differentiation techniques in the metaphase chromosomes of a great diversity of organisms, a large number of papers were written which reported its presence, variability, molecular composition, direct and indirect effects on the karyotype, putative function, etc. (see Mizianty, 1984a,b, 1985; John, 1988; Hennig, 1999). One of the most basic, but by no means the least important aspects of HC research, is the study of its distribution in blocks or bands throughout the karyotype. Analysis of band distribution patterns can make it possible to verify whether the bands are preferentially located in certain chromosomal regions, suggesting that the HC has a functional purpose.

### HETEROCHROMATIN VARIABILITY

The analysis of HC distribution patterns in the chromosome complement of angiosperms in general is hindered by the particularly high variability of HC added to the variability of other karyotype parameters. The comparison of HC band patterns of different species should take into consideration the following aspects: 1) the HC is not homogeneous, varying qualitatively and quantitatively between

species; 2) within a single species, polymorphism for the number and size of bands is frequent; 3) the amount of HC varies regardless the amount of euchromatin or the nuclear DNA content; 4) both HC and euchromatin can suffer large changes in a relatively short time; 5) different staining techniques may reveal different HC fractions; 6) very different species may present simultaneous differences in chromosome number, size and morphology, as well as in the amount, composition and distribution of HC (Greilhuber, 1982; Vosa, 1985; John, 1988; Sumner, 1990).

The functional and evolutionary meaning of HC may not be the same for all species, and a single distribution pattern for all angiosperms may not exist, only tendencies or preferential patterns for different genomes and karyotype architectures.

In spite of these difficulties in establishing general models, some authors draw attention to distribution patterns that seem to occur very frequently. Heitz (1933) was the first to observe that, in each species, the HC seemed to be distributed non-randomly in proximal, interstitial and telomeric regions. In *Vicia faba*, for instance, he observed that HC was preferentially located in interstitial regions, while in *Allium cepa* its distribution was preferentially terminal. After performing chromosome analysis of several species of plants and animals, Heitz (1957) considered that such an equilocal distribution is a universal rule.

Lima de Faria (1976a,b, 1983) considered that heterochromatin blocks observed by "cold starvation" or by C-banding, as well as chromomeres, knobs, secondary constrictions and other chromosomal markers and DNA sequences, would occupy preferential sites within the *chromosome field* (the chromosome region between centromere and telomere where specific DNA sequences and chromosome phenomena occur in a specific order). Greilhuber (1979), however, re-examined the available data on the distribution of "cold starved" HC and found no preferential regions.

Later, Loidl and collaborators (Loidl, 1983; Greilhuber and Loidl, 1983; Schweizer and Loidl, 1987) drew attention to the fact that in many species HC blocks tend to be distributed in the arms of different chromosomes at an equal distance starting from the centromere (see also Bennett, 1982). In such cases, the chromosomes would tend to exhibit *equidistant* bands (e.g., on the telomeres of similar-sized arms and in the corresponding interstitial regions

of the longest arms), while, according to Heitz, the distribution of bands would be preferentially equilocal (e.g., on the telomeres of short and long arms). These tendencies would result in different karyotype patterns, with equilocalization producing a generalized banding pattern in all chromosomes of a complement (regardless of size and centromeric index variation), while equidistance would generate different banding patterns in asymmetrical karyotypes and identical patterns in symmetrical karyotypes.

#### HETEROCHROMATIN CHARACTERIZATION

One of the difficulties in understanding HC distribution is the fact that bands of a single karyotype may be composed of different types of HC, i.e., unrelated families of repetitive DNA with independent distribution patterns (Flavell, 1982). Therefore, it is important to separately evaluate the distribution of each HC type by cyto-molecular techniques, as has been done for rye (Appels *et al.*, 1978), *Scilla* (Deumling and Greilhuber, 1982), barley (Brandes *et al.*, 1995), *Aegilops* (Badaeva *et al.*, 1996) and a few other organisms. Working with conventional cytological techniques, only the HC associated with the nucleolar organizer region (NOR-HC) can be specifically identified, due to secondary constrictions or by silver nitrate impregnation. More reliable recognition of the NOR-HC has recently been achieved by *in situ* hybridization, mainly with pTa71 probe, which is able to localize rDNA 18S-5.8S-26S sites in every kind of plant (see, e.g., Galasso *et al.*, 1996a; Hizume *et al.*, 1992) and at least in some animals (Pendás *et al.*, 1993). An alternative way of distinguishing some HC types is to stain the chromosomes with fluorochromes that have a preferential affinity for AT- or GC-rich DNA. This staining can differentiate the HC in only a few groups, allowing the easy observation of their distribution in the chromosomes.

HC may be revealed by different cytological techniques, with identical or very similar results (Vosa, 1976b; Lozano *et al.*, 1990; Berg and Greilhuber, 1993). Conventional staining techniques may sometimes show heteropycnotic regions that form the so-called *prophase condensation patterns* (Fukui and Mukai, 1988; Ikeda, 1988; Benko-Iseppon and Morawetz, 1993). Such regions have often been confused with HC patterns which may be very different from each other (see Morawetz, 1981a; Guerra, 1988a). The HC revealed by C-banding seems to correspond to the most general conception of HC, coinciding with the sites of late replicating DNA and tandem organized satellite DNA (Baumann, 1971; Appels *et al.*, 1978; Deumling and Greilhuber, 1982; Cortés and Escalza, 1986; Schubert and Rieger, 1991). However, the use of different C-banding techniques may result in very different banding patterns. In *Citrus*, for example, HC blocks were found mostly at terminal regions (Guerra, 1985, 1993a; Miranda *et al.*, 1997; Pedrosa *et al.*, 2000), although Ito *et al.* (1993), using a different technique, found exclusively proximal HC blocks. In corn, Aguiar-Perecin and Vosa (1985) reported

C-bands in terminal and subterminal chromosomal regions, coinciding in number and position with the knobs. However, Molina (1981) found only proximal bands in all the chromosomes, while Chow and Larter (1981) found proximal and terminal bands. On the other hand, Carvalho and Saraiva (1993), using another technique (KHG banding), found numerous interstitial bands. In all these cases the bands were clear and unequivocal, and the differences in these results were certainly due to the procedures used, which revealed different chromatin fractions.

Besides the C-banding technique, staining with base-specific fluorochromes has been recognized as a reliable method of distinguishing some types of HC in plants (Vosa, 1970, 1976b; Schweizer, 1976). The fluorochromes quinarine (Q), Hoechst 33258 (H) and 4'-6-diamidino-2-phenylindole (DAPI) preferentially stain AT-rich HC (Q+, H+ and DAPI+ bands, respectively) while mitramycin (MM) and chromomycin A3 (CMA) preferentially stain GC-rich (MM+ or CMA+) regions (Schweizer, 1976; Sumner, 1990). These same fluorochromes may also negatively stain AT-poor or GC-poor HC blocks, respectively, whereas double staining with two fluorochromes of different base specificity (e.g., CMA/DAPI, CMA/H or MM/DAPI) may highlight staining differences (Schweizer, 1976, 1981; Kenton, 1991). In addition, non-fluorescent DNA-ligands, like dystamycin A, may also be used to intensify the CMA or DAPI staining (Schweizer, 1983; Fuchs *et al.*, 1998). However, not all AT-rich or GC-rich HC reacts equally to these fluorochromes (see e.g., Schwarzach and Schweizer, 1982; Kenton, 1991; Bennett *et al.*, 1995) and some C-banded positive regions react neutrally with fluorochromes, i.e., they fluoresce with the same brightness as euchromatin (Morawetz, 1986a,b; Röser, 1994; Galasso *et al.*, 1996b; Cuellar *et al.*, 1996). Other C-bands, fluoresce with the same intensity when stained with fluorochromes with different base-specificities, such as CMA and DAPI (Loidl, 1983; Guerra, 1989; Okada, 1991; Berg and Greilhuber, 1993). All these HC types were considered here as *neutral* bands.

In this paper the C-band distribution patterns of 105 species, belonging to 58 dicotyledons and 32 monocotyledons genera, were compared in order to identify general patterns of HC distribution or any preferential sites in which it may occur. Only the results obtained with the C-banding techniques most used in plants (Marks, 1975; Schwarzach *et al.*, 1980; Gill *et al.*, 1991) will be considered. These techniques are based on chromosome hydrolysis with 45% acetic acid, or dilute hydrochloric acid, followed by denaturation in barium hydroxide, treatment with 2x SSC and Giemsa staining. Information about the reactivity to base-specific fluorochromes is included for 58 of these species. The main questions focused on are: 1) Is there a preferential distribution of C-bands in the proximal, interstitial or telomeric regions? 2) Are band distribution patterns influenced by the average chromosome size of the karyotypes? 3) Is there any relationship between GC-rich or AT-rich HC bands and their chromosomal location? 4) Is the

occurrence of generalized equiloal bands influenced by their base composition, their chromosomal position, or the average chromosome size?

#### MATERIAL ANALYZED: CRITERIA FOR DATA SELECTION

To evaluate the variability of HC distribution patterns among angiosperms, data were compiled for 105 species belonging to 90 genera (Table I). Each species name was preceded by the first three letters of its family name to help recognize the taxa, along with diploid number, average chromosome size, band position in the chromosomes, occurrence and position of generalized bands and consulted references.

Species were selected for inclusion in this list simply because they were published or unpublished author's

original data or because they were cited in papers available to the author. To avoid overrepresenting the banding patterns of more widely studied plant groups, such as *Triticum*, *Hordeum*, etc., only a single species of each genus was considered. However, in genera with large karyotype variation, either in the amount or the distribution of HC, like *Capsicum* and *Scilla*, two or more representatives were included. In these cases preference was given to the species with extreme HC patterns and to those about which data was available for both C-band and fluorochrome staining. The references used were always those with good photographic documentation which was compatible with the description in the text, the photographic data generally being more highly valued than the text description. Papers with no photographic data were not considered.

The sample was divided into three groups according

**Table I** - C-band distribution in plant karyotypes with small, medium and large average chromosome size (ACS). Generalized bands (G) may be located at the telomeric (T), interstitial (I) and proximal (P) regions. Family names are indicated by the three first letters (ANA = Anacardiaceae, ANN = Annonaceae, etc). Numbers after species names identify the fluorochromes used: 1, DAPI; 2, quinacrine; 3, Hoechst 33258; 4, CMA. Heterochromatin with main base composition identified but without any indication of fluorochrome used was stained with CMA/DAPI. NOR associated bands are identified by number, position (T, I or P), and AT or GC richness; base composition not defined = ?; number in brackets are NOR sites without C-bands. Estimated values are indicated by ca. (circa).

Family-species	2n	ACS	Heterochromatin distribution				G	References
			T	I	P	NOR		
<b>Small chromosomes (&lt;3.0 μm)</b>								
ANA - <i>Anacardium occidentale</i>	40	1.1	4GC	-	-	2T-GC	-	Gitai and Guerra (1999)
ANN - <i>Porcelia goyazensis</i>	18	ca.2.8	4	-	6	2T	-	Morawetz (1984)
ANN - <i>Guatteria schlechtendaliana</i>	28	ca.2.0	-	-	28N	2GC	P	Morawetz and Waha (1985)
ARA - <i>Hedera helix</i>	48	ca.2.5	-	2GC	48N	2I-GC	P	König <i>et al.</i> (1987)
ARE - <i>Johannesteijsmannia altifrons</i>	34	ca.2.0	-	-	34AT	2T-GC	P	Röser (1994)
ARI - <i>Thottea siliquosa</i>	26	ca.1.8	2	-	26	-	P	Morawetz (1985)
AST - <i>Artemisia capillaris</i>	18	2.4	6	-	18	-	P	Mendelak and Schweizer (1986)
AST - <i>Emilia sonchifolia</i>	10	2.5	-	-	10N	2T-GC	P	Guerra and Nogueira (1990)
								Guerra, M. (unpublished data)
AST - <i>Helianthus annuus</i>	34	ca.2.8	-	-	34N	6T-GC	P	Cuellar <i>et al.</i> (1996)
BIG - <i>Jacaranda macrantha</i>	36	ca.2.3	4	-	36	4T	P	Morawetz (1982)
BRA - <i>Arabidopsis thaliana</i> <sup>1</sup>	10	ca.1.5	-	-	10AT	4AT(?)	P	Ambros and Schweizer (1976); Maluszynska and Heslop-Harrison (1991)
BRA - <i>Raphanus sativus</i>	18	ca.3.0	-	-	18	2T	P	Hirai <i>et al.</i> (1995)
BRA - <i>Sinapsis alba</i>	24	ca.2.0	4	-	24	-	P	Geber and Schweizer (1988)
CAP - <i>Lonicera caprifolium</i>	18	ca.2.0	14	-	18	-	P	Benko-Iseppon and Morawetz (1993)
CUC - <i>Cucumis sativus</i>	14	ca.1.9	24	-	8	2I	T	Ramachandran <i>et al.</i> (1985)
EUP - <i>Manihot esculenta</i>	36	1.8	4GC	2N	-	4T-GC	-	Carvalho and Guerra (1999)
FAB - <i>Arachis hypogaea</i>	40	2.13	6	10	40	2I	P	Cai <i>et al.</i> (1987); Fernández and Krapovickas (1994)
FAB - <i>Cicer arietinum</i>	16	ca.2.8	-	2GC + 4AT	16AT	2I-GC	P	Galasso <i>et al.</i> (1996a)
FAB - <i>Medicago sativa</i>	16	ca.2.3	12	16N	16N	2I-GC	I+P	Masoud <i>et al.</i> (1991); Calderini <i>et al.</i> (1996)
FAB - <i>Phaseolus calcaratus</i>	22	1.4	-	-	22	2T	P	Zheng <i>et al.</i> (1991)
FAB - <i>Vigna ambacensis</i>	20	ca.2.8	8GC	-	20N	-	P	Galasso <i>et al.</i> (1996b)
FAB - <i>Vigna sesquipedalis</i>	22	2.0	6	3	22	2T	P	Zheng <i>et al.</i> (1991)
MAG - <i>Liriodendron tulipifera</i>	38	ca.1.3	28	-	7	-	T	Morawetz (1981a)
MON - <i>Peumus boldus</i>	78	2.9	-	-	78	12T	P	Morawetz (1981b)
ORC - <i>Orchis coriophora</i> <sup>3</sup>	36	ca.2.5	20AT	-	-	(12I)	-	D'Emérico <i>et al.</i> (1996)
RUT - <i>Boenninghausenia albiflora</i>	20	ca.1.8	-	-	20	-	P	Guerra (1985b)
RUT - <i>Citrus hystrix</i>	18	ca.2.0	ca.13	-	ca.10	2T	-	Guerra (1985b)
RUT - <i>Coleonema pulchrum</i>	34	ca.1.5	-	-	12	2T	-	Guerra (1985b)

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Table I - Continued

Family-species	2n	ACS	Heterochromatin distribution				G	References
			T	I	P	NOR		
RUT - <i>Glycosmis pentaphylla</i>	54	ca.2.0	-	-	-	6T-GC	-	Guerra <i>et al.</i> (in press); Guerra, M. and Santos, K.G.B. (unpublished data)
RUT - <i>Murraya paniculata</i>	18	ca.2.3	18GC	-	4N	2P-GC	T	Guerra (1985b); Guerra <i>et al.</i> (in press)
SAP - <i>Cardiospermum grandiflorum</i>	20	2.4	12AT	-	-	4T-GC	-	Hemmer and Morawetz (1990)
SAP - <i>Serjania subdentata</i>	24	2.2	12AT	-	-	6T-GC	-	Hemmer and Morawetz (1990)
SOL - <i>Solanum tuberosum</i>	24	ca.2.7	10	12	18	2I	-	Pijnacker and Ferwerda (1984)
VEL - <i>Vellozia pattens</i>	16	ca.0.8	-	-	16N	4T-GC	P	Melo <i>et al.</i> (1997)
<b>Medium chromosomes (&gt;3.0 &lt;5.0 µm)</b>								
ANN - <i>Annona muricata</i>	14	ca.3.0	4N	-	6AT+8N	2P-GC	P	Morawetz (1986a)
ANN - <i>Rollinia pulchrinervis</i>	42	4.0	-	-	18N+20AT	4T-GC	P	Morawetz (1986b)
ARE - <i>Coccothrinax litoralis</i>	36	ca.3.9	-	2 AT	36N	2T-GC	P	Röser (1994)
ARE - <i>Schippia concolor</i>	36	ca.3.9	-	-	36N	2P-GC	P	Röser (1994)
AST - <i>Crepis capillaris</i>	6	4.7	4	8	4	2T	T	Siljak-Yakovlev and Cartier (1979)
AST - <i>Lactuca sativa</i>	18	ca.3.2	-	20	18	4T	P	Koopman <i>et al.</i> (1993)
CAP - <i>Viburnum opulus</i>	18	ca.4.0	14GC	ca.10GC	-	2T-GC	T	Benko-Iseppon and Morawetz (1993)
COM - <i>Callisia sp.</i>	24	ca.5.5	-	-	24	-	P	Jones and Kenton (1984)
COS - <i>Costus pulverulentus</i>	18	ca.3.5	-	-	18AT	2T-GC	P	Guerra (1988a)
FAB - <i>Lathyrus aphaca</i> <sup>2</sup>	14	3.9	12AT	18AT	-	(2I)	-	Rees and Hazarika (1969); Ünal <i>et al.</i> (1995)
FAB - <i>Lathyrus tingitanus</i> <sup>2</sup>	14	4.5	2AT	2GC	14GC	(2I)	I+P	Rees and Hazarika (1969); Ünal <i>et al.</i> (1995)
FAB - <i>Sesbania tetraptera</i>	12	4.6	-	-	12N	4T-GC	P	Forni Martins <i>et al.</i> (1994); Forni Martins and Guerra (1999)
FAB - <i>Vicia johannis</i>	14	ca.4.1	12	-	-	2I	T	Ramsay (1984)
FAB - <i>Vicia lutea</i>	14	ca.4.7	14	18	-	2T	T+I	Ramsay (1984)
GOO - <i>Scaevola taccada</i>	16	ca.4.0	-	-	16N	2T-GC	P	Morawetz (1986a)
HYA - <i>Fortunaria arida</i> <sup>2</sup>	34	3.6	29AT	3AT	-	-	T	Fernandez and Daviña (1991)
HYA - <i>Muscari comosum</i>	18	ca.3.0	-	8AT	18AT	2GC(?)	P	Lozano <i>et al.</i> (1990)
IRI - <i>Eleutherine bulbosa</i>	12	3.3	1N	4AT	12AT	2P-GC	P	Guerra (1988b)
ORC - <i>Cephalanthera longifolia</i>	32	3.4	-	2AT	32AT	2I-GC	P	Schwarzacher and Schweizer (1982)
ORC - <i>Psymorchis pusilla</i>	12	3.8	-	-	12N	-	P	Felix and Guerra (in press)
POA - <i>Milium effusum</i>	28	3.3	44	-	10	4I	T	Bennett and Thomas (1991)
POA - <i>Zea mays</i>	20	ca.4.5	8	20	-	2I	I	Aguar-Perecin (1985); Aguar-Perecin and Vosa (1985)
POA - <i>Zingiber biebersteiniana</i> <sup>1,2,3</sup>	4	ca.4.9	8N	10N	4AT	2GC	T+ I+P	Bennett <i>et al.</i> (1995)
RUB - <i>Genipa americana</i>	22	ca.4.0	ca.24GC	ca.12GC	ca.10GC	2T-GC	T	Guerra (1993b); Pierozzi and Mendaçolli (1997)
RUT - <i>Dictamnus albus</i>	36	ca.3.5	72	-	-	-	T	Guerra (1985b)
SOL - <i>Capsicum annuum</i>	24	4.1	ca.24GC	2GC	-	4T-GC	T	Moscone <i>et al.</i> (1993, 1996); Moscone, E. (personal communication)
SOL - <i>Capsicum pubescens</i>	24	3.9	2AT	2GC	24N	4T-GC	T+P	Moscone <i>et al.</i> (1993, 1996); Moscone, E. (personal communication)
<b>Large chromosomes (&gt;5.0 µm)</b>								
ADO - <i>Adoxa moschatellina</i>	36	5.8	45	1	-	7T	T	Greilhuber (1979)
ALL - <i>Allium fistulosum</i> <sup>2</sup>	16	ca.11.5	30GC	4N	ca.14N	2T-GC	T+P	Kamizyô and Tanaka (1978)
ALL - <i>Allium subvillosum</i>	28	ca.5.4	26GC	19GC	28GC	6T-GC	T+P	Jamilena <i>et al.</i> (1990)
ALL - <i>Nothoscordum cf pulchellum</i>	10	14.3	-	-	-	2T-GC	-	Felix, L.P. and Guerra, M. (unpublished data)
ALL - <i>Nothoscordum fragrans</i>	19	ca.16.0	2AT	6GC	-	5T-GC	-	Sato and Yoshioka (1984); Crosa (1996)
ALL - <i>Tulbaghia pulchella</i> <sup>2</sup>	12	10.5	11GC	-	-	(2P)	T	Dyer (1963); Vosa (1970)
ALS - <i>Alstroemeria magnifica</i>	16	7.4	2	11	-	6I	-	Buitendijk <i>et al.</i> (1998)
ALS - <i>Alstroemeria ligtu</i>	16	14.9	14	ca.46	16	6P	T+I+P	Buitendijk <i>et al.</i> (1998)
AST - <i>Anacyclus "coronatus"</i>	18	6.0	ca.20	4	18	6T	T+P	Schweizer and Ehrendorfer (1976)
AST - <i>Anacyclus depressus</i>	18	6.1	-	-	18	4T	P	Schweizer and Ehrendorfer (1976)

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Table I - Continued

Family-species	2n	ACS	Heterochromatin distribution				G	References
			T	I	P	NOR		
AST - <i>Artemisia judaica</i>	16	6.0	16	-	13	6T	T+P	Mendelak and Schweizer (1986)
AST - <i>Crepis praemorsa</i>	8	9.2	13	ca.15	-	-	T	Siljak-Yakovlev and Cartier (1979)
AST - <i>Crepis vesicaria</i>	8	6.2	-	-	8	2T	P	Guerra (1982)
AST - <i>Hypochoeris brasiliensis</i>	8	7.8	-	-	6	2I+2C	P	Ruas <i>et al.</i> (1995)
AST - <i>Santolina</i> sp.	36	6.1	ca.66	-	ca.32	4T	T+P	Guerra, M. (unpublished data)
BOR - <i>Buglossoides purpureoerulea</i> <sup>2</sup>	16	ca.5.9	20GC	20GC	-	(4I)	T+I	D'Amato <i>et al.</i> (1981)
COM - <i>Gibasis karwinskyana</i> <sup>1,4</sup>	10	11.2	18AT	10AT	-	2T-GC	T+I	Kenton (1978); Kenton (1991)
FAB - <i>Vicia faba</i>	12	ca.9.5	-	ca.46AT	2AT	2I-GC	I	Greilhuber (1975); Fuchs <i>et al.</i> (1998)
FAB - <i>Vicia melanops</i>	10	ca.11.6	8AT	ca.20AT	-	3I-GC	T	D'Amato <i>et al.</i> (1980)
HYA - <i>Hyacinthoides italica</i>	16	11.2	12	ca.24	16	2P	I+P	König and Ebert (1997)
HYA - <i>Ornithogalum tenuifolium</i> <sup>2</sup>	6	5.1	-	-	-	2P-GC	-	Vosa (1997)
HYA - <i>Prospero autumnale</i>	12	ca.7.7	-	8AT	12AT	2GC	P	Ebert <i>et al.</i> (1996)
HYA - <i>Puschkinia scilloides</i>	10	5.5	6	-	-	2T+4I	-	Greilhuber and Speta (1976)
HYA - <i>Scilla mischtschenkoana</i>	12	10.0	1AT	ca.14 AT	-	4T-GC	I	Greilhuber and Speta (1978); Deumling and Greilhuber (1982)
HYA - <i>S. persica</i>	8	13.8	-	-	-	2T	-	Greilhuber and Speta (1978)
HYA - <i>S. siberica</i>	12	15.0	10GC	5GC	12AT	2P-GC	T+P	Greilhuber and Speta (1978); Deumling and Greilhuber (1982)
LIL - <i>Bulbine alata</i>	28	5.1	36	4	28	2T	T+P	Watson (1988)
LIL - <i>Lilium canadense</i>	24	ca.16.0	-	50	24	4T	I+P	Smyth <i>et al.</i> (1989)
LIL - <i>Tulipa gesneriana</i> <sup>2,3,4</sup>	24	ca.11.0	36	ca.36	-	-	T+I	Blakey and Vosa (1982)
ORC - <i>Cypripedium segawai</i>	20	ca.13.8	24AT	61AT	20AT	(2P)	T+I+P	Kondo <i>et al.</i> (1994); Hoshi <i>et al.</i> (1995)
PAE - <i>Paeonia tenuifolia</i>	10	19.1	-	-	10N	6T-GC	P	Schwarzacher-Robinson (1986)
POA - <i>Dasyphyrum villosum</i>	14	ca.6.0	ca.26AT	ca.26AT	ca.14AT	2T-GC	T+I+P	Cremonini <i>et al.</i> (1994); Pignone <i>et al.</i> (1995)
POA - <i>Festuca rubra</i>	42	ca.5.5	ca.70	-	-	-	T	Bailey and Stace (1992)
POA - <i>Hordeum vulgare</i>	14	ca.11.7	ca.20	ca.62	-	4I	T+I	Kakeda <i>et al.</i> (1991)
POA - <i>Secale cereale</i> <sup>3</sup>	14	9.1	28AT	ca.40AT-N	14N	2I-GC	T+I+P	Sarma and Natarajan (1973); Mukai <i>et al.</i> (1992)
POA - <i>Triticum longissimum</i>	14	ca.9.0	28	92	14	4I	T+I+P	Friebe <i>et al.</i> (1993)
RAN - <i>Aconitum sanyoense</i>	16	ca.6.0	4GC +14AT	4AT	6AT-2N	2T-GC	T	Okada (1991)
RAN - <i>Anemone blanda</i> <sup>1</sup>	16	ca.12.0	-	40AT	-	2T	I	Marks and Schweizer (1974); Hagemann <i>et al.</i> (1993)
RAN - <i>Helleborus foetidus</i> <sup>2</sup>	32	ca.7.4	-	-	-	6T-GC	-	D'Amato and Bianchi (1989)
RAN - <i>Nigella damascena</i>	12	ca.11.0	-	-	12	2T	P	Marks (1975)
SAL - <i>Azima tetracantha</i>	22	9.2	ca.28N	ca.6N	22N	2I-GC	T+P	Guerra (1989)
SOL - <i>Cestrum fasciculatum</i>	16	ca.6.0	-	ca.7GC +16AT	14N	4T-GC	I+P	Berg and Greilhuber (1993)
SOL - <i>Cyphomandra luteoalba</i>	24	6.1	10	46	-	2T	I	Pringle and Murray (1993)
TRI - <i>Paris tetraphylla</i>	10	22.7	8	6	10	2T	T+P	Miyamoto and Kurita (1990)

to average chromosome size, based on the fact that the heteropycnotic characteristics of the chromatin of species with small, medium and large chromosomes are frequently different, resulting in different structural types of interphase nuclei (Delay, 1949; Guerra, 1987). Small chromosomes (<3.0  $\mu\text{m}$ ) usually have arreticulate or pro-chromosome nuclei, which present early condensed proximal areas in prophase, e.g., heterochromatic blocks. On the other hand, large chromosomes (>5.0  $\mu\text{m}$ ) usually have reticulate or eurenticulate nuclei, with more or less homogeneous chromosome condensation. Medium-sized chromosomes (>3.0 <5.0  $\mu\text{m}$ ) display semi-reticulate nuclei with irregular and variable chromosome condensation (Delay, 1949; Guerra, 1987). This correlation is most evident between DNA amount and nuclear interphase type (Barlow, 1977).

However, since no DNA amount estimates were available for many of the species included, nuclear interphase type was correlated with average chromosome size.

The chromosome size was preferentially based on measurements supplied by the referred authors, although in the many cases where the chromosome size was not referred to in the paper, but a scale bar was presented in the photos or idiograms, a length estimation was made with the help of the scale. In these species the calculated chromosome size are more inaccurate, since such photographs show the banding pattern, but not necessarily with a representative chromosome size. In Table I these values are indicated as circa (ca.).

Whenever possible, the chromosome size estimated from conventionally stained cells was adopted, since the

procedure used for C-banding or fluorochrome staining can apparently change the chromosome size. Schwarzacher and Schweizer (1982), for instance, reported an increase in size of up to 50% for chromosomes of *Cephalanthera* cells after C-banding, in comparison with those stained with Feulgen, while Moscone *et al.* (1996) observed that *Capsicum* chromosomes stained with fluorochrome could be up to 1/3 larger than conventionally stained ones. On the other hand, *Cyphomandra* chromosomes treated with C-banding techniques were reduced to a third of the size of those stained with orcein (Pringley and Murray, 1993). In some cases, as in *Arabidopsis thaliana* and *Arachis hypogaea*, chromosome measurements made by other authors with conventional staining were used, since it was considered that any errors resulting from intraspecific polymorphism would be smaller than those arising from the measurement of a single cell. In any case, the chromosome size presented is just an approximation of the real size, used to separate the species into the three groups.

In this paper the position of the bands was classified as *proximal* when located in the proximal region or immediately after the primary constriction, as *telomeric* when located in the terminal regions of the chromosome, and as *interstitial* or *intercalar* when they occupied neither of the chromosome arm extremities. When the band occupied the whole chromosome arm, it was classified as telomeric or proximal depending on the dominant band pattern in the karyotype.

Concerning HC reactivity in relation to base-specific fluorochromes, the bands were classified as AT, GC or N (for neutral), depending on how they reacted with fluorochromes that preferentially stain DNA sequences rich in AT or GC. Species analyzed with the fluorochromes DAPI, quinacrine, Hoechst 33258 or CMA are indicated in Table I by the numbers 1, 2, 3 or 4, respectively, after the species name. The other species with an indication of main base composition but without a number were stained with CMA plus DAPI.

The bands associated with the NOR can involve the secondary constriction and/or the chromatin adjacent to this region, and for this kind of band the description in the papers was more important than photographs, since secondary constrictions have very variable expression and may not be expressed in that cell (Sato *et al.*, 1980).

## RESULTS AND DISCUSSION

In spite of the great variability known in HC distribution patterns, drastic and discontinuous changes within a related group of species are not common. In most of the genera in which data were available for at least five species, the number of bands and the HC amount varied but the general pattern was relatively well conserved, as in *Anacyclus* (Schweizer and Ehrendorfer, 1976), *Secale* (Bennett *et al.*, 1977), *Vigna* (Zheng *et al.*, 1991; Galasso *et al.*, 1996b) and *Citrus* (Guerra, 1993a; Miranda *et al.*,

1997). The diversity was much higher in genera with karyotypes exhibiting numerous interstitial bands, like *Anemone* (Marks and Schweizer, 1974), *Scilla* (Greilhuber, 1982), and *Tulipa* (Blakey and Vosa, 1982). Data from very different, non-angiosperm taxa, like some gymnosperm or bryophyte genera, also show similar tendencies (see Tanaka and Hizume, 1980; MacPherson and Filion, 1981; Newton, 1986a,b; Davies *et al.*, 1997).

Considering Table I as a representative sample of angiosperms, the genera and species most frequently analyzed with C-banding techniques are those with large chromosomes, like *Triticum*, *Gibasis*, *Allium*, *Scilla*, etc. Most of these genera are monocotyledons, whereas those with small chromosomes and only a few studied species are mainly dicotyledons. The diploid number of the sampled species is quite representative of angiosperms, although very high numbers are not represented. No relationship between chromosome number and any HC characteristic was found.

### Frequency of telomeric, interstitial, and proximal C-bands

Table II presents the frequency of species with telomeric, interstitial or proximal C-bands, in relation to chromosome size. In general, C-bands were slightly more frequent in the proximal region than in other regions. Considering only the karyotypes with HC restricted to a single chromosome region one can observe that it was found in 33 of the 105 species, 23 of which had proximal bands, although these numbers may contain some bias. The frequency of telomeric bands may be overestimated due to the common occurrence of NOR-HC in this region, which is not always identified with this technique and may be wrongly counted as common C-bands. On the other hand, the very reduced frequency of interstitial HC blocks in small chromosomes may be partly due to technical difficulties in localizing these bands on the arms of very small chromosomes.

The distribution of C-bands seems to depend at least partly on chromosome size. Proximal bands, in general, were more common in karyotypes with small chromosomes, decreasing in frequency with increasing chromo-

**Table II** - Number and percentage of species analyzed in Table I with telomeric, interstitial or proximal bands.

Average chromosome size	Number of species in each size category (%)	Band position		
		Telomeric	Interstitial	Proximal
Small	34 (32.4)	20 (58.8)*	7 (20.6)	28 (82.4)
Medium	27 (25.7)	16 (59.3)	16 (59.3)	19 (70.4)
Large	44 (41.9)	30 (68.2)	30 (68.2)	25 (56.8)
Total	105	66	53	72

\*The sum of percentages in each size category is over 100 because some species have bands in two or three positions.

some size (Table II). Furthermore, karyotypes with only proximal bands were found in 11 of the 34 species with small chromosomes but in only 12 of the 71 species with large and medium-sized chromosomes, suggesting that this region develops or conserves C-bands more frequently.

The predominance of proximal bands in karyotypes with small chromosomes is more evident in the distribution of *generalized equilocal bands*, considered here as those bands occurring in the same region in at least 80% of the chromosomes of a karyotype. In general, such bands were less common in species with small chromosomes (Table III). However, among 24 species with small chromosomes and generalized equilocal bands, 21 showed generalization in the proximal region, three in the telomeric region and only one in the interstitial region. Such contrast is far less evident in species with medium or large chromosomes (Table III). Very often generalized proximal bands

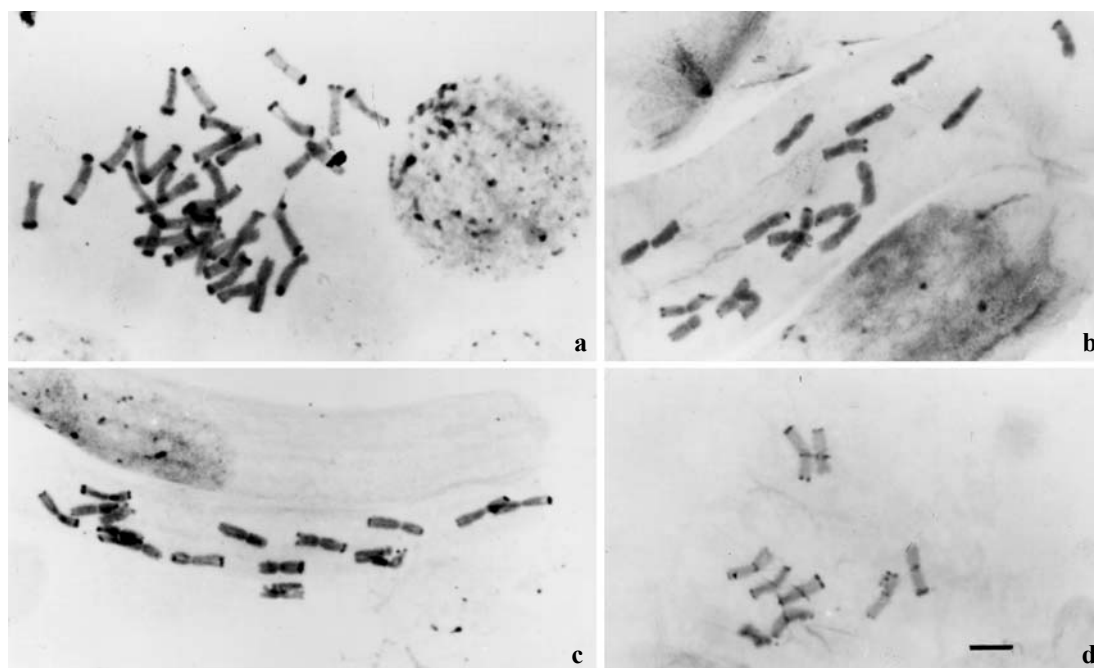
are largely conserved throughout a genus, as in *Crepis* (Ikeda, 1988) or *Sesbania* (Forni-Martins and Guerra, 1999). The frequencies of generalized bands in Table III are overestimated due to the *a priori* selection of species with the maximum number of bands in those genera with a single representative, although this bias was the same for every chromosome size class.

Generalized proximal HC has also been observed in several species only when using modified C-banding methods, as in onion (Fiskesjo, 1974; Cortés and Escalza, 1986), or after *in situ* hybridization, as in *Vigna unguiculata* (Guerra *et al.*, 1996), or after sequential CMA/DAPI staining and C-banding, as in *Cardiospermum grandiflorum* (Hemmer and Morawetz, 1990). In some other species proximal C-bands have been observed only occasionally. Figure 1 illustrates the occurrence of occasional C-bands in *Santolina* (Asteraceae-Anthemidae). In two diploid spe-

**Table III** - Number and percentage of species with generalized equilocal bands in different chromosomal positions for chromosomes of different sizes (based on Table I).

Average chromosome size	Number of species		Band position		
	Total	With generalized bands	Telomeric	Interstitial	Proximal
Small	34	24 (70.6)*	03 (12.5)*	01 (4.2)	21 (87.5)
Medium	27	26 (96.3)	11 (42.3)	04 (15.4)	16 (61.5)
Large	44	36 (81.8)	23 (63.8)	16 (44.4)	23 (63.8)
Total	105	86 (81.9)	37 (43.0)	21 (24.4)	59 (65.1)

\*The sum of percentages for each size category is over 100 because some species have generalized bands in two or three positions.



**Figure 1** - C-banded metaphase chromosomes of *Santolina* species. **a**, *Santolina* sp. ( $2n = 36$ ). **b**, *S. rosamarinifolia* ( $2n = 18$ ). **c**, **d**, *S. chamaecyparissus* ( $2n = 18$ ). Note proximal bands in most chromosomes of the tetraploid species and at the partial metaphase in **d**. Bar in **d** represents 5  $\mu\text{m}$ .

cies, *S. rosamarinifolia* and *S. chaemacyparissus*, only telomeric bands were found, whereas in a tetraploid, *Santolina* sp., telomeric and proximal bands were always observed (Figure 1a-c). However, in a single slide of *S. rosamarinifolia*, proximal bands were found in all chromosomes (Figure 1d). Such occasional results are certainly not rare, but usually go unreported. These data suggest that the occurrence of any detectable kind of proximal HC is much higher than that observed here, and it may be a common feature of all angiosperm chromosomes (see also Vosa, 1985).

Another interesting aspect is that the occurrence of generalized bands in the interstitial region was not only much lower but almost always accompanied by generalization in another region, since 15 of 20 species with generalized interstitial bands also presented generalization in the telomeric or proximal region.

#### NOR associated heterochromatin

Secondary constrictions were not observed or not mentioned in only 14 of the 105 species of Table I. They are usually more difficult to visualize when they are very small or terminally located in the chromosomes (Guerra *et al.*, 1997; Pedrosa *et al.*, 2000). In general, the secondary constrictions were located in the short arm, close to the telomere, as observed by Lima de Faria (1976a), and the NOR-HC was located distally, occupying one or both sides of the constriction.

The NOR-HC is clearly differentiated from other HC types because it contains rDNA genes, although it may also contain repeated sequences not related to the NOR, such as the 120-bp repeat unit of rye (Cuadrado and Jouve, 1994). Analysis of rDNA site distribution by *in situ* hybridization shows that they are always present in the NOR and often in other regions where secondary constrictions have not been located. In *Vigna unguiculata*, for instance, only one or two chromosomes with secondary constrictions were observed, whereas 10 rDNA sites were revealed by FISH (Guerra *et al.*, 1996), eight of them terminally located. NOR-HC often stains differently from other HC types (Sato *et al.*, 1979; Guerra, 1988a) and in extreme cases it does not react positively after C-banding or fluorochrome staining, as in *Tulbaghia pulchella* (Vosa, 1970), *Buglossoides purpureo-caerulea* (D'Amato *et al.*, 1981), *Cypripedium segawai* (Kondo *et al.*, 1994; Hoshi *et al.*, 1995), *Lathyrus aphaca*, *L. tingitanus* (Ünal *et al.*, 1995) and *Orchis coriophora* (D'Emerico *et al.*, 1996).

#### Bands observed with fluorochromes

The most commonly used fluorochromes were CMA and DAPI, and among the 58 species analyzed with fluorochromes in Table I, 44 were stained with this fluorochrome combination, almost always using the double-staining tech-

nique proposed by Schweizer (1976). In some cases, besides CMA and DAPI, other fluorochromes were also used as primary stains (Hoshi *et al.*, 1995; Sato and Yoshioka, 1984) or as counterstains (Deumling and Greilhuber, 1982; Jamilena *et al.*, 1990; Cuellar *et al.*, 1996). Quinacrine was the third most frequently used fluorochrome (10 species), followed by Hoechst 33258.

Bands observed with fluorochromes were also positively stained after C-banding, although not all C-bands were differentiated by fluorochromes. In some species, however, a small number of fluorescent bands were found at sites where C-bands were not detected, as in *Aconitum sanyoense* (Okada, 1991) and *Cestrum fasciculatum* (Berg and Greilhuber, 1993). This fact, together with the apparent lack of NOR associated C-bands in some species, indicates once again that not all heterochromatin is detectable with the most usual C-banding techniques (see, Vosa, 1985).

The relationship between the intensity of the brightness of a fluorochrome stained chromatin and its base composition was originally established for cells not treated with C-banding or any other treatment promoting DNA denaturation-renaturation or preferential extraction of the euchromatin (Casperson *et al.*, 1969; Schweizer, 1976; Sumner, 1990). However, some authors have used fluorochrome staining after the C-banding technique, obtaining bright bands which may not be an indication of AT- or GC-richness (Silva and Guerra, 1998). Bennett *et al.* (1995), for instance, after chromosome staining with quinacrine or DAPI of *Zingiber biebersteiniana* (n = 2), observed fluorescent bands only in the proximal region of one chromosome pair. Using C-banding plus the same fluorochromes, they observed proximal DAPI+ bands in both chromosome pairs and a terminal band in one of them, coinciding with Giemsa stained C-bands. These same DAPI+ bands were also observed after *in situ* hybridization. Vosa (1976a,b) observed that the NOR-HC of some species was positive for Hoechst and quinacrine after C-banding but negative for the same fluorochrome without pretreatment. Such fluorescent bands obtained only after C-banding were considered as neutral in Table I.

The most characteristic HC detected with fluorochromes was the NOR-HC, almost always CMA+ and DAPI-. The reaction intensity of CMA with this HC was variable. Most frequently it was more intense than with other GC-rich bands, but in *Muscari comosum* and *Psymorchis pusilla* it was faintly stained or neutral. Interestingly, in *Arabidopsis thaliana* the NOR associated HC was apparently positive after staining with DAPI (Maluszynska and Heslop-Harrison, 1991; Ross *et al.*, 1996), although its reaction with CMA is unknown. In this case, staining with CMA or mithramycin is important to verify whether or not a small CMA+ region exists in the secondary constriction itself or very close to it. Positive bands for DAPI or Hoechst, very close to the NOR, have been reported in several other species (Kenton, 1991; Cremonini *et al.*, 1994).



### Distribution of fluorochrome bands

Most species have at least one pair of CMA+ bands, since all of them have at least one pair of NORs (Moravetz, 1986a; Röser, 1994; Guerra *et al.*, in press). In some species there are one or two pairs of CMA+ blocks at a very similar chromosome position to that of the NOR, although no secondary constriction is observed associated with them. This occurs in *Hedera helix* (König *et al.*, 1987), *Cicer arietinum* (Galasso *et al.*, 1996a) and *Hypochoeris chilensis* (Cerbah *et al.*, 1995). However, after *in situ* hybridization with rDNA probes, these CMA bands are also often labelled, suggesting that it is the same type of HC (Galasso *et al.*, 1996a; Cerbah *et al.*, 1998).

In Table I, excluding the NOR bands, AT-rich blocks were found in 29 species, GC-rich in 19 species and neutral blocks in 23 species. Note that the total number of AT- or GC-rich sites per karyotype is not under consideration, but rather the number of species exhibiting at least one such site per chromosomal region. AT-rich sites were more frequently found at the interstitial region in species with medium and large chromosome size (Table IV). Particularly in the case of bimodal karyotypes with long acrocentrics and small metacentrics or acrocentrics, interstitial bands were almost always AT-rich, as in some *Brimeura* species (Vosa, 1979), *Fortunatia arida*, *Muscari comosum*, *Ornithogalum tenuifolium*, *Eleutherine bulbosa* and *Cephalanthera longifolia* (Table I). A noteworthy exception was *Nothoscordum fragrans*, with interstitial bands that were positive for mithramycin and chromomycin A3 and negative for quinacrine (Sato and Yoshioka, 1984). Other karyotypes basically composed of long acrocentrics, like *Anemone blanda* and *Vicia faba* (Table I), were also characterized by interstitial AT-rich bands.

Among GC-rich bands, some were certainly non-identified rDNA sites, suggesting that the proportion of spe-

cies with AT-rich HC is even higher. Nevertheless, in some taxa, GC-rich bands are very diversified and highly dominant or even exclusive, as in the genus *Capsicum* (Moscone *et al.*, 1996), in the subfamily Aurantioideae (Guerra *et al.*, in press) or in the family Lemnaceae (Geber, 1989).

Equilocal bands almost always stain in the same way with fluorochromes, suggesting that most of these bands are made up of the same or very similar repeats. Analyses in several organisms with *in situ* hybridization of specific satellite DNA fractions have shown that such repeats usually have equilocal distribution (Appels *et al.*, 1978; Badaeva *et al.*, 1996; Brandes *et al.*, 1995). In *Aegilops*, for instance, a specific satellite sequence was observed in the terminal and subterminal regions of all species investigated, as well as in other chromosomal regions (Badaeva *et al.*, 1996). In some species, two or more unrelated satellite DNAs have been observed occupying the same equilocal sites (Brandes *et al.*, 1995), which may explain those sites that stain positively with different base specific fluorochromes.

### CONCLUDING REMARKS

The analysis of C-band patterns in a sample of over one hundred angiosperm karyotypes with different chromosome sizes showed that HC is not randomly distributed but rather that it is preferentially located. The NOR-HC is a notorious example, with a very marked distribution at subtelomeric sites. This HC type is very frequently, but not always, positively stained by C-banding techniques and by fluorochromes with high affinity for GC-rich chromatin. The remaining HC is mainly AT-rich and, regardless of its DNA base composition, it has a generalized equilocal distribution. In small chromosomes the bands are conspicuously preferentially located in proximal regions. Such generalized proximal bands may be related to the high frequency of prochromosomal nuclei in species with small chromosomes and low DNA content (Delay, 1949; Barlow, 1977; Guerra, 1987).

The bands seen here showed a tendency to be preferentially distributed in some specific chromosome regions, regardless of distance from the centromere. Thus, generalized telomeric C-bands were found in similar- and dissimilar-sized arms of a single karyotype, as in *Paris tetraphylla* (Miyamoto and Kurita, 1990), *Aconitum sanyoense* (Okada, 1991) and *Fortunatia arida* (Fernández and Daviña, 1991). Likewise, NOR-HC was terminally located in karyotypes with similar-sized arms, as in *Allium subvillosum* (Jamilena *et al.*, 1990), or different-sized arms, as in *Paeonia tenuifolia* (Schwarzacher-Robinson, 1986). Generalized interstitial C-bands can be also equidistant, as in *Anemone blanda* (Marks and Schweizer, 1974) and *Lathyrus tingitanus* (Ünal *et al.*, 1995), or, more frequently, occupy different positions in interstitial regions, as in several species of *Lilium* (Smyth *et al.*, 1989) and *Cyphomandra* (Pringle and Murray, 1993).

**Table IV** - Distribution of C-bands with different base composition in karyotypes of small, medium and large chromosomes (based on Table I).

Band position	Main base composition	Average chromosome size			Total
		small	medium	large	
Telomeric	GC	04	04	06	14
	AT	03	04	08	15
	N	-	03	01	04
Intercalar	GC	02	05	05	12
	AT	01	07	11	19
	N	02	02	03	07
Proximal	GC	-	03	01	04
	AT	03	07	05	15
	N	08	08	06	22
Total	GC	06	12	12	30
	AT	07	18	24	49
	N	10	13	10	33

One of the important assumptions in the heterochromatin dispersion model proposed by Schweizer and Loidl (1987) is that interstitial bands in long arms are equidistant to the terminal bands of short arms. Such symmetrical distribution was attributed to HC amplification and transposition occurring during mitotic interphase between non-homologous chromosomes showing Rabl orientation. In the present sample, such interstitial bands corresponding to non-homologous terminal HC were sometimes observed, although often along with several non-equidistant interstitial bands, as in *Gibasis karwinskyana* (Kenton, 1978, 1991) and *Secale cereale* (Mukai *et al.*, 1992). Chromosomes of *Allium subvillosum*, for instance, exhibited telomeric bands in all short arms and interstitial bands in all long arms, but, according to the idiogram of Jamilena *et al.* (1990), the interstitial bands were located at distances almost always larger than the longest of the short arms. Actually, an exact equidistance is not expected. Since different chromosome arms may have different condensation/decondensation patterns (Okada, 1975; Fukui and Mukai, 1988), the distance between one band and the centromere in non-homologous arms may be quite different in metaphase and in interphase.

These data suggest that generalized bands are not just an accident due to chromatin proximity of more or less equidistant non-homologous regions but most probably are due to the functional or structural similarity of these regions. The equilocal or equidistant chromatin "contamination" by the same type of repetitive DNA sequence may be facilitated, but not determined, by Rabl orientation. This assumption is supported by HC distribution in species with holocentric chromosomes, which are free from the influence of Rabl orientation. Karyotypes of *Drosera* (Sheik and Kondo, 1995, 1996) and *Rhynchospora* (Vanzela *et al.*, in press), genera with holocentric chromosomes and belonging to widely different angiosperm families, exhibit HC distribution patterns similar to those with monocentric chromosomes. In both genera, generalized equilocal C-bands were found, which also gave identical fluorochrome reactions. In several species of *Rhynchospora*, Vanzela *et al.* (1998) observed the occurrence of multiple rDNA sites always distributed in the terminal regions. Similarly, satellite DNA sequences with generalized equilocal distribution were observed in the holocentric chromosomes of the peach potato aphid (Spence *et al.*, 1998; Mandrioli *et al.*, 1999). Therefore, HC equidistribution may not depend fundamentally on Rabl orientation and band-centromere distance, but rather on some structural or functional similarity of each chromosomal region that makes them equally receptive to the installation and/or amplification of the same or similar DNA sequence (Fry and Salser, 1977; Flavell, 1982).

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