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Giemsa C-banded karyotypes in *Capsicum* (*Solanaceae*)*

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Abstract: Giemsa C-banding is applied for the first time in *Capsicum*, allowing preliminary karyotype differentiation of six diploid species. Comparison of interphase nuclei and heterochromatic C-bands reveals striking differences between taxa and contributes to their taxonomic grouping. Therefore, C-banding appears to be a powerful tool for the cytogenetics and karyosystematics of the genus. Banding patterns are characterized by the omnipresence of centromeric bands and a variable number of smaller to larger distal bands, with the addition of intercalary bands in some cases. Satellites are always C-positive. Relationships between species and possible trends of karyotype evolution are discussed, with special reference to the origin of x = 13 from x = 12 and the increase of heterochromatin, regarded as advanced features.

Capsicum is a New World genus of *Solanaceae-Solaneae* with about 25 species of which 5 are widely cultivated for their economic value as vegetables and spices (cf. HEISER & SMITH 1953, HUNZIKER 1979, ESHBAUGH 1983). The taxonomy of *Capsicum* is still difficult and partly confused (HUNZIKER 1971, PICKERSGILL & al. 1979). Provisionally, the cultivated species and their wild relatives are subdivided according to their predominant corolla colour into a "white-" and a "purple-flowered group" (JENSEN & al. 1979; MCLEOD & al. 1979, 1983; ESHBAUGH 1983; PICKERSGILL 1988, 1991).

Karyological data have been obtained for more than half of the species (cf. PICKERSGILL 1977, 1991; MOSCONE 1989, 1990, 1992, 1993). Chromosome numbers are mostly 2n = 2x = 24, including the cultivated taxa, but 2n = 2x = 26 has also been recorded.

So far, constitutive heterochromatin patterns in the chromosomes of *Capsicum* have not been widely studied, since only SHOPOVA (1966) has presented some very provisional data on three species of the genus. The innovative approach of Giemsa

* Chromosome studies in *Capsicum* (*Solanaceae*), III. For the first and the second part see MOSCONE (1990, 1993).

C-banding has been applied up to now in only 19 species of *Solanaceae* in the following genera: *Cestrum* (BERG & GREILHUBER 1992 a, b), *Cyphomandra* (PRINGLE 1990), *Hyoscyamus* (TYAGI & GILL 1990), *Nicotiana* (MOURAS 1982, MOURAS & al. 1986), *Petunia* (DIETRICH & al. 1981, WIJSMAN & al. 1983), and *Solanum* (PIJNACKER & FERWERDA 1984, MOSCONE 1989).

Thus, a program of Giemsa C-banding for *Capsicum* was devised with the objectives of: (1) improving the karyological characterization of species, varieties and cultivars; (2) relating Giemsa C-banding patterns to classical karyotypes and DNA values; (3) contributing to a more natural classification; (4) exploring karyoevolutionary mechanisms and trends; (5) increasing our knowledge of genetic resources among cultivated and wild taxa.

In this first contribution we report on preliminary Giemsa C-banding studies on six taxa of *Capsicum*. Two of them are wild woody species, *C. chacoënse* A. T. HUNZ. and *C. parvifolium* SENDT., the former growing in the Chaco forest (Paraguay, Bolivia, Argentina; HUNZIKER 1950) and the latter in northeastern Brazil (Ceará, R. Grande do Norte, Paraíba, Pernambuco, Piauí, Bahia), Colombia (Magdalena) and Venezuela (Aragua, Sucre, Carabobo). The remaining four are herbs and include one wild SE Brazilian species: *C. campylopodium* SENDT., and three cultivated taxa: *C. annuum* L. var. *annuum*, probably of Mexican origin (PICKERSGILL 1971); *C. baccatum* L. var. *pendulum* (WILLD.) ESHBAUGH, possibly of Bolivian origin (MCLEOD & al. 1982); *C. pubescens* RUIZ & PAV., primarily with an Andean distribution but known only from cultivation, and possibly domesticated in S Peru or Bolivia (cf. PICKERSGILL 1971; MCLEOD & al. 1979, 1982).

Material and methods

The origin of the plant material studied is shown in Table 1, together with essential karyological and morphological features. Voucher specimens have been verified by A. T. HUNZIKER and are deposited in the herbarium of Museo Botánico de Córdoba, Argentina (CORD).

Somatic Giemsa C-banded chromosomes were observed in root tip squashes obtained from seed germination. For breaking dormancy, 500–1000 ppm gibberellic acid (GA_3) was used (ELLIS & al. 1985). Root tips were pretreated in a saturated solution of p-dichlorobenzene in water for 2 h at room temperature, fixed in 1 : 3 glacial acetic acid/96% ethanol mixture and stored in the refrigerator for a minimum of 12 h. The Giemsa C-banding procedure was performed according to SCHWARZACHER & al. (1980), except that the root apices were softened in enzymatic solution (1% pectinase plus 1% cellulase) at 37 °C for only 15–30 min.

Cut-out karyograms were prepared from microphotographs of single metaphase plates by arranging the chromosomes first in respect to their increasing arm ratio, and then according to their decreasing length within each group. The arm ratio (r = l/s) was used to classify the chromosomes as recognized by LEVAN & al. (1964): m = metacentric (r = 1.00–1.69), sm = submetacentric (r = 1.70–2.99), st = subtelocentric (r = 3.00–6.99) and t = telocentric (r = 7.00–∞). For the satellites we have used the terminology of BATTAGLIA (1955) with some modifications: microsatellite (diameter smaller than the chromosome diameter) and macrosatellite (diameter the same as the chromosome diameter and of equal or smaller size than of the corresponding chromosome arm). The satellite lengths were always added to the lengths of the respective arms, but the lengths of the secondary constrictions were excluded. The data presented in this paper are based on a limited number of taxa and single individuals and thus should be considered as preliminary. A larger

Table 1. List of *Capsicum* species studied, provenance, karyotype features, corolla colour, and life form

Species	Provenance and voucher specimen*	2n	Karyotype formula (n) and length in [μm]	Corolla colour	Life form
C. chacoënse	Argentina, Prov. Córdoba, Dept. Calamuchita, Falda del Sauce (w). EAM 104	24^{x}	10 m + 1 m (sat) + 1 st (sat)^{x} [40.68]	white	perennial
C. parvifolium	Brazil, Est. Bahia, Munic. Senhor do Bomfin and Itiuba, cultivated in Viçosa (w). ATH 25233	24•	11 m + 1 m (sat)• [52.46]	white or purple (both with green-yellowish spots in the throat)	perennial
C. annuum var. *annuum*	Argentina, Prov. Córdoba, Córdoba (c). EAM 193	24°	10 m + 1 sm (sat) + 1 st° [48.93]	white	annual
C. baccatum var. *pendulum*	Argentina, Prov. Córdoba, Córdoba (c). EAM 192	24°	10 m + 1 m (sat) + 1 st (sat)° [45.08]	white (throat with green-yellowish spots)	annual
C. pubescens	Bolivia, bought at market place in Salta (Argentina), cultivated in the Botanical Garden, University of Vienna (c). EAM 198	24°	10 m + 1 m (sat) + 1 st (sat)° [46.61]	purple (white inner throat)	annual
C. campylopodium	Brazil, Est. Minas Gerais, Munic. Caratinga, Mata de Jaó (w). ATH 25130	26^{+}	10 m + 1 sm (sat) + 1 sm + 1 st^{+} [49.39]	white (throat with yellow spots)	annual

* *ATH* A. T. Hunziker; *EAM* E. A. Moscone; *w* natural habitat/wild origin; *c* cultivar. References for chromosome numbers, karyotype formulae and lengths: Moscone (1989^{+}, 1990^{x}, 1993•, and unpubl.°).

number of taxa, each represented by several individuals are being examined in the course of ongoing research.

Karyotype analyses

Chromosome numbers, karyotype formulae and lengths for each taxon studied are included in Table 1 and are briefly described in the text. This information is followed by the results of Giemsa C-banding analyses which are illustrated by photographs of mitotic plates, interphase nuclei and cut-out karyograms (Figs. 1–3).

All taxa analysed are characterized by the presence of centromeric C-bands in every chromosome; they are weakly stained and just visible as faint pairs of dots in *C. chacoënse*, *C. parvifolium*, and *C. annuum* var. *annuum*, but strongly represented (sometimes appearing as lines) in *C. baccatum* var. *pendulum*, *C. pubescens*, and *C. campylopodium* (Figs. 1, 3). The satellites are always C-positive, the adjacent secondary constrictions being negatively C-banded.

Short comparisons with classical karyotype information available from the literature close the presentation of each species. In many cases, the chromosome terminology followed by other authors is vague, and chromosome measurements or illustrations are sometimes lacking. Therefore, comparisons with our results described according to the widely accepted nomenclature of LEVAN & al. (1964; see above), are only tentative. The sequence of taxa corresponds to their possible affinities.

Capsicum chacoënse. This species with 2n = 24 is represented by a karyotype with two satellited chromosome pairs, a large m (1) and a small st (12), and with 10 m pairs (2–11) of decreasing size (Table 1). There are only small amounts of C-heterochromatin (Figs. 1 a, 3 a). Some chromosomes have small terminal C-bands on one arm or, rarely, on both (pairs 10 and 11). Intercalary C-bands are completely absent. The macrosatellites of pair 1 and the microsatellites of pair 12 are the only regions strongly stained with Giemsa. The former are prominent and appear as large dark chromocentres in interphase nuclei (Fig. 2 a). The rather similar morphology and length of most of the chromosomes and their limited banding do not allow a clear identification of the homologous metacentric pairs 2–9.

The present Giemsa C-banded karyotype of *C. chacoënse* agrees very well with a karyotype constructed from chromosomes stained with alcoholic hydrochloric acid carmine which was obtained from the same accession (P_1: MOSCONE 1990: fig. 2 A–B). The macrosatellites on pair 1 are contrasted even more clearly with the microsatellites of pair 12; also, pairs 10 and 11 are better marked by Giemsa C-staining. The structural diversity among populations of this species has been documented by MOSCONE (1990) in his comparison of accession P_1 with P_2 which carries the macrosatellites on the smallest m pair. There are other reports on variation between cytotypes of *C. chacoënse*, having one or two satellited chromosome pairs. In the latter, the short "acrocentric" ($\cong$ st) satellited pair reported by PICKERSGILL (1977) may correspond to our pair 12, while the large satellited pair illustrated by LIMAYE & PATIL (1989) could possibly match our pair 1. Otherwise, the classical karyotypes presented by these and earlier authors agree in principle with our data for this species. Thus, CHENNAVEERAIAH & HABIB (1966) and OHTA (1962) both reported two satellited pairs, although the latter disregarded the presence of any st pair.

Capsicum parvifolium. This species has a 2n = 24 karyotype with one m satellited (12) and 11 other m pairs (1–11) of rather similar length (Table 1). In most of the chromosomes small terminal C-bands occur in either the short or the long arm, being absent from pairs 2 and 10 (Figs. 1 b, 3 b). None of the chromosomes has intercalary bands. The most conspicuous Giemsa-staining regions are the short arm telomeres of pair 4, the long arm telomeres of pair 8 and the macrosatellites of pair 12. The rather weak banding of chromosomes in this taxon is also reflected in its interphase nuclei, the chromocentres being small and few in number (Fig. 2 b).

Giemsa C-banding confirms the tentative chromosome homologies suggested on the basis of a previous conventional karyotype analysis in the only sample of *C. parvifolium* studied so far (Moscone 1993), although each chromosome pair can now be better identified. In particular, pair 12 is clearly distinct because of its strongly Giemsa C-stained macrosatellites. Moreover, some pairs, otherwise easily misplaced due to their similar size and arm ratio, are clearly separated by their C-banding (pair 1 vs. 2, 4 vs. 5, 8 vs. 9).

Capsicum annuum* var. *annuum. The 2n = 24 karyotype of the cultivar studied has one sm satellited (11), one st (12) and 10 m pairs (1–10) of decreasing size (Table 1). Distinguishing features of many chromosomes are the presence of distal C-bands in just one arm, usually the short one (Figs. 1 c, 3 c). Only one chromosome, a member of pair 5, has terminal bands in both telomeres, making this pair heteromorphic with respect to its C-banding pattern. Most of the terminal C-bands are small but those on the short arms of pair 4 are comparatively large and strongly stained, as are the macrosatellites of pair 11. Pairs 2, 7, 9, and 12 are not banded at the telomeric regions. Intercalary C-bands are present only on the short arms of pairs 9 and 11, close to the secondary constrictions in the latter. In interphase nuclei four chromocentres are distinguishable from the remainder by their larger size (Fig. 2 c). These correspond to the large C-bands on chromosome pairs 4 and 11.

The C-banded karyotype described agrees very well with a classical karyotype from the same population (Moscone, unpubl.). C-banding facilitates the distinction of many chromosome pairs and the establishment of homologies which are otherwise difficult to resolve (e.g., pair 4 vs. 5, 6 vs. 7, 9 vs. 10). Furthermore, the macrosatellites of pair 11 are also brought out much more clearly.

Intraspecific karyotype variation, mostly concerning the number of st pairs (1–2, rarely 3 or none) and satellited pairs (1–2, exceptionally 3) has been well documented for *C. annuum* (cf. Chennaveeraiah & Habib 1966; Pickersgill 1971, 1977, 1991; Limaye & Patil 1989). The karyotype of the accession analysed here is very similar to one of the cytotypes described by Pickersgill (1971, 1977, 1991) with two "acrocentric" pairs named by her (1991) "chromosome race 2". From the illustrations of her contributions (1971: fig. 2, 1991: fig. 2 c) it can be inferred that one of such pairs (which is satellited) is clearly sm, the other st. Our present data also agree completely with the cultivar karyotypes reported by Ohta (1962), Shopova (1966), Carluccio & Saccardo (1977), and Ming & Dehua (1986). On the other hand, our sample exhibits some karyotype differences from that examined by Moscone (1993), in which the satellited pair is m (but with a comparatively high arm ratio).

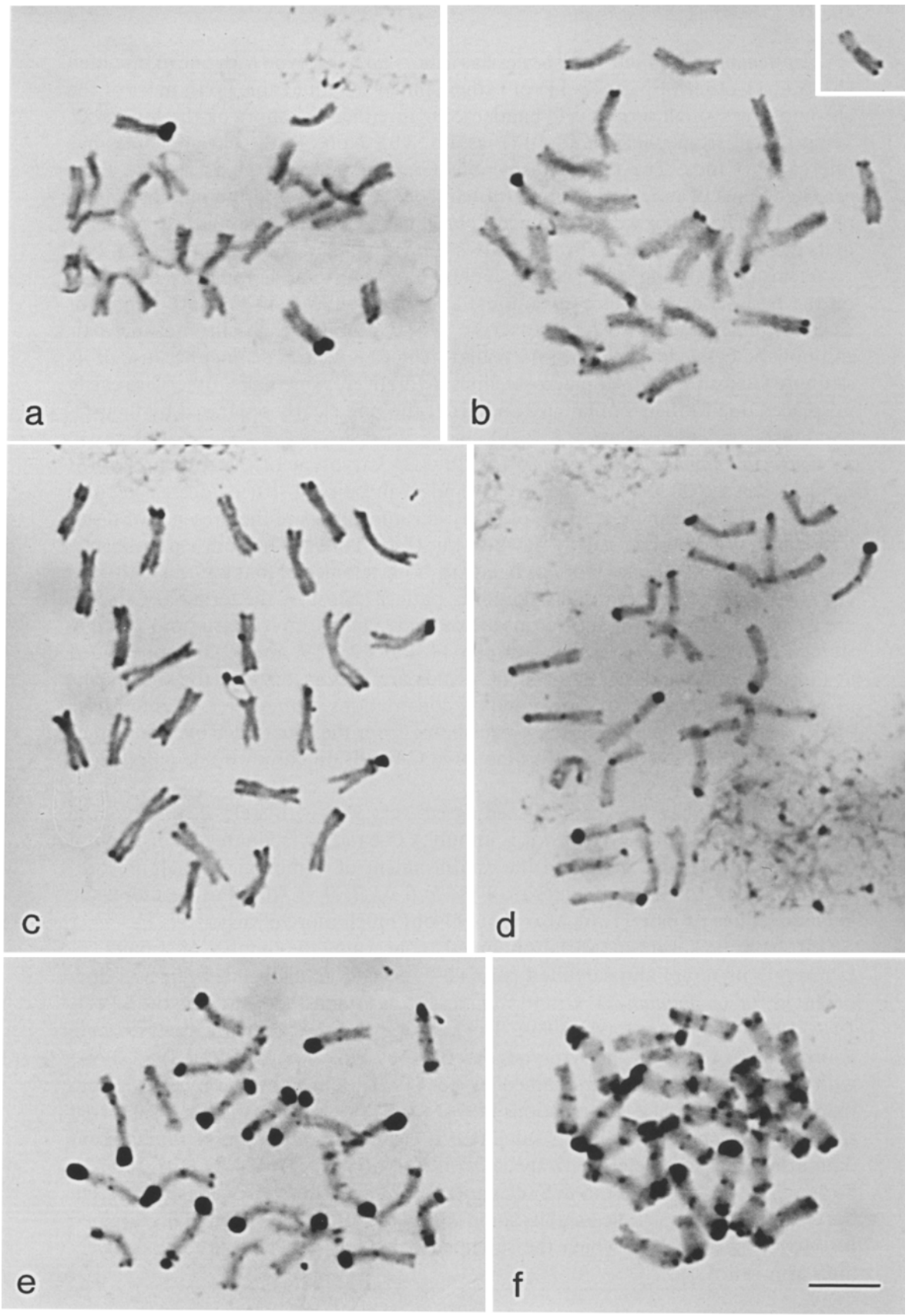


Fig. 1. Giemsa C-banded somatic metaphases of *Capsicum* species. *a C. chacoënse*, 2n = 24. *b C. parvifolium*, 2n = 24. *c C. annuum* var. *annuum*, 2n = 24. *d C. baccatum* var. *pendulum*, 2n = 24. *e C. pubescens*, 2n = 24. *f C. campylopodium*, 2n = 26. Bar: 5 µm

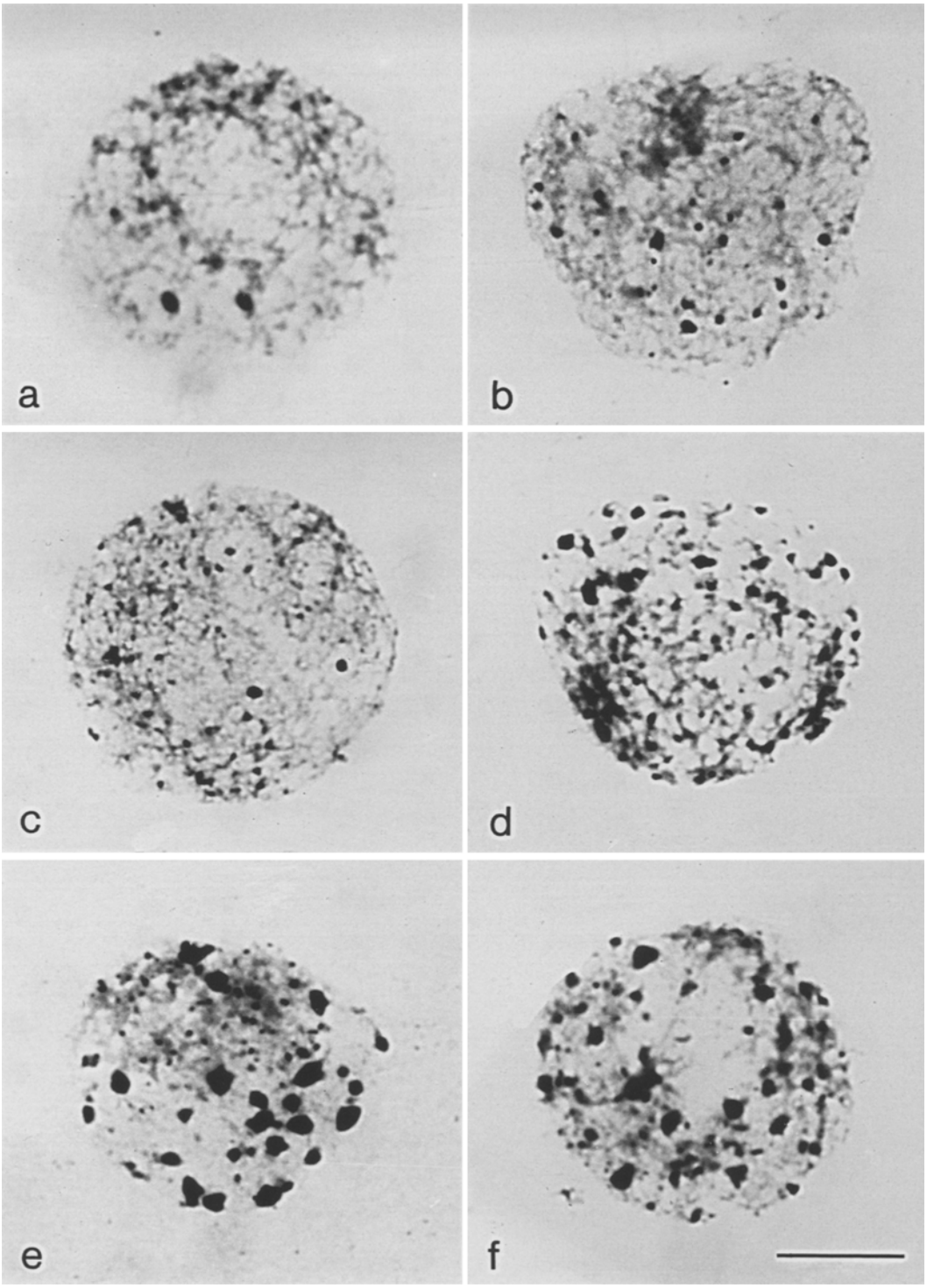
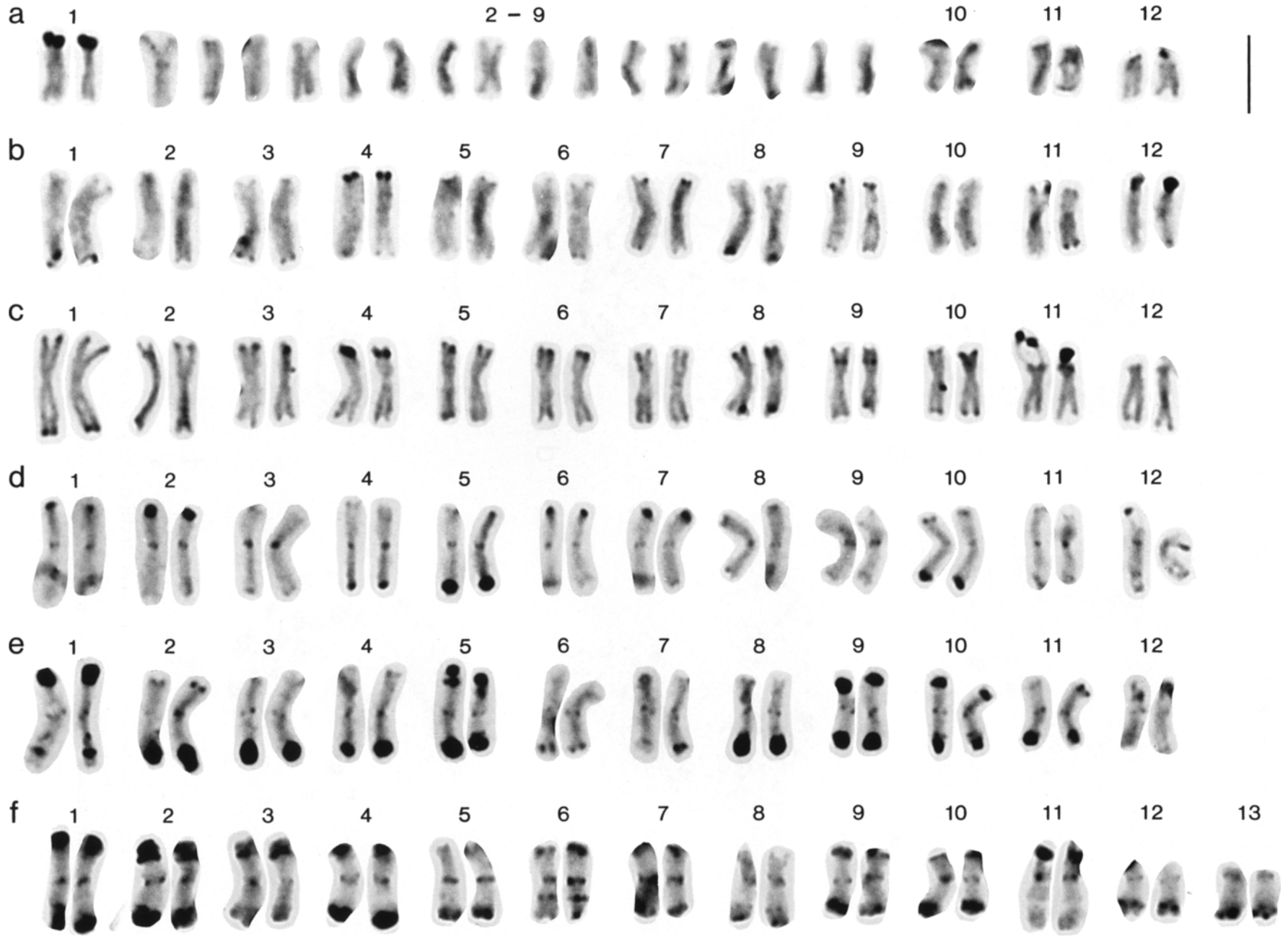


Fig. 2. Giemsa C-stained interphase nuclei showing interspecific variation in number and size of chromocentres in *Capsicum* species. *a C. chacoënse. b C. parvifolium. c C. annuum* var. *annuum. d C. baccatum* var. *pendulum. e C. pubescens. f C. campylopodium.* Bar: 10 µm


a
1
2 – 9
10
11
12
b
1
2
3
4
5
6
7
8
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c
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***Capsicum baccatum* var. *pendulum*.** The sample examined has a 2n = 24 karyotype composed of 11 m pairs (1–11) of rather similar size and 1 st pair (12), the pairs 2 and 12 being satellited (Table 1). Telomeric C-bands are present, with the exception of pair 9 (Figs. 1 d, 3 d). In general, only one chromosome arm – either the short or the long one – is terminally banded, but with the size of the C-positive segments and the intensity of staining varying among pairs. Intercalary C-bands are completely absent. The largest terminal bands are on the short arms of pair 2 (macrosatellites) and 7, as well as on the long arms of pairs 5 and 10. Homomorphy is high in the C-banding pattern of homologous chromosomes. Interphase nuclei exhibit distinct dark chromocentres of different sizes (Fig. 2 d).

The present C-banded karyotype of *C. baccatum* var. *pendulum* agrees well with a classical karyotype of the same accession (MOSCONE, unpubl.). After conventional staining there are doubtful homologies, but these are resolved by Giemsa C-banding due to better marking of the chromosomes (i.e., pair 4 vs. 5, 6 vs. 7, 9 vs. 10).

Karyotype heterogeneity between populations, particularly as regards satellited pairs, has been recorded for the variety *pendulum* and the rest of the species (cf. CHENNAVEERAIAH & HABIB 1966, PICKERSGILL 1977, LIMAYE & PATIL 1989). Most of these authors describe cytotypes with one or two m satellited pairs (rarely 3–4: CHENNAVEERAIAH & HABIB 1966), but PICKERSGILL (1977, 1991) also reports a cytotype with two satellited pairs, one of them "acrocentric" ($\cong$ st) as in our accession. Otherwise, there is general agreement with previous data obtained by OHTA (1962), CHENNAVEERAIAH & HABIB (1966), CARLUCCIO & SACCARDO (1977), PICKERSGILL (1977), and KURIACHAN (1981); only LIMAYE & PATIL (1989) disregard the st pair in their sample of *C. baccatum* var. *pendulum* (sub *C. pendulum*).

***Capsicum pubescens*.** The 2n = 24 karyotype of the stock studied has 11 m pairs (1–11) of decreasing size and 1 st pair, the pairs 5 and 12 being satellited (Table 1). The striking feature of this accession is its large amount of C-heterochromatin which, in general, is asymmetrically distributed on the two arms of each chromosome (Figs. 1 e, 3 e). Most of the chromosomes have a large block of heavily stained telomeric C-heterochromatin in one arm (usually the long one), and a minute terminal band or an unbanded telomere in the other. Pairs 5, 9, and 10 show prominent terminal C-bands in both arms, whereas pairs 6 and 7 possess relatively small C-heterochromatic segments. Pair 7 is unique in the lack of terminal bands in both arms. There are intercalary C-bands located in a subterminal position of

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Fig. 3. Diploid karyotypes of *Capsicum* species showing the Giemsa C-banding pattern. *a C. chacoënse*, chromosomes of pairs 2–9 are arranged in order of decreasing length because pairing of homologues was uncertain, pairs 1–11 are m and pair 12 is st, pairs 1 and 12 carry satellites. *b C. parvifolium*, pairs 1–12 are m, pair 12 bears satellites. *c C. annuum* var. *annuum*, pairs 1–10 are m, pair 11 is sm and pair 12 is st, pair 11 has satellites. *d C. baccatum* var. *pendulum*, pairs 1–11 are m and pair 12 is st, pair 2 and 12 carry satellites. *e C. pubescens*, pairs 1–11 are m and pair 12 is st, pairs 5 and 12 bear satellites. *f C. campylopodium*, pairs 1–10 are m, pairs 11–12 are sm and pair 13 is st, pair 11 has satellites. Satellites are always on short arms. Numbers refer to homologous chromosome pairs for each karyogram, although the numbers do not necessarily denote homoeology between the different complements. Bar: 5 µm

the long arms of pairs 1, 7, and 10, and the short arms of pair 5. In the last, the interstitial C-positive segments are close to the secondary constrictions. The high number of dark chromocentres in interphase nuclei, most of them being large (Fig. 2 e), correlates well with the number and size of bands on the metaphase chromosomes. Heteromorphism in C-bands between homologous chromosomes is limited to some differences in size (terminal bands of pairs 2 and 4) or intensity of staining (subterminal bands of pair 7).

The present C-banded karyotype of *C. pubescens* corresponds well with a karyotype obtained from chromosomes stained with alcoholic hydrochloric acid carmine of material from the same source (MOSCONE, unpubl.). As all pairs of the complement exhibit distinctive C-banding patterns, reliable identification of pairs with similar length and arm ratio (i.e., pair 2 vs. 3, 6 vs. 7, 9 vs. 10) is possible by Giemsa C-staining. Furthermore, positive C-staining of satellites allows easy recognition of pairs 5 and 12.

Karyotype variation between populations, mostly concerning the satellited chromosomes, has been reported for this species (cf. CHENNAVEERAIAH & HABIB 1966, PICKERSGILL 1977, LIMAYE & PATIL 1989). Our results agree with the karyotype with two satellited pairs, one of them "acrocentric" (≅ st), described by PICKERSGILL (1977). The cytotypes presented by OHTA (1962) and SHOPOVA (1966) are also very similar, although the former lists only one satellited pair, probably corresponding to our pair 5. CHENNAVEERAIAH & HABIB (1966) and LIMAYE & PATIL (1989) also report on different cultivars with 1–2 (exceptionally 3) satellited pairs, but none of them is an st.

Capsicum campylopodium. This species with 2n = 26 (Table 1) is represented by a karyotype with 10 m pairs (1–10) of decreasing length, 2 sm pairs – one larger and satellited (11), the other smaller (12) – and 1 small st pair (13). The diploid complement of the population analysed has a complex C-banding pattern (Figs. 1 f, 3 f). Diversity in C-bands results in striking differences among pairs. Terminal and intercalary C-bands of different size occur throughout the complement, the former being more frequent. Symmetrical C-heterochromatin distribution on both arms characterizes the two longest pairs (1 and 2). Double bands, one terminal and one subterminal occur in some pairs, either on one arm (pairs 8, 12, and 13) or on both (pairs 2 and 3). C-banding heteromorphism between homologous chromosomes is largely absent, but a terminal band present in one of the members of pair 6 does not appear in the other, which is a remarkable heteromorphy. Interphase nuclei exhibit numerous chromocentres in a wide range of sizes (Fig. 2 f).

The C-banded karyotype of *C. campylopodium* presented here agrees completely with a karyotype derived from alcoholic hydrochloric acid carmine karyotype staining obtained from the same sample (P_2: MOSCONE 1989), where only the satellited pair 11 and the two smallest pairs (12 and 13) are easily distinguishable. In contrast, separation between m pairs with similar length and arm ratio (e.g., 2 vs. 3, 5 vs. 6) is possible by Giemsa C-banding, which also produces additional distinct markers for every chromosome pair of the complement.

In this species, MOSCONE (1989) has found another strikingly different cytotype in a population of different provenance (P_1), which exhibits predominantly sm chromosomes.

Discussion

The study of Giemsa C-banded chromosomes has been an important step forward in the cytogenetics of many wild and cultivated plant groups. Such studies have been attempted in *Capsicum* (cf., PICKERSGILL 1991), but so far have failed because of technical difficulties. Earlier experiments with cold-sensitive chromosome segments (SHOPOVA 1966) have been unsatisfactory, but could be repeated with improved methods (cf. BERG & GREILHUBER 1992 a, b).

The present observations demonstrate that C-banding techniques are a powerful tool in the cytogenetics and karyosystematics of *Capsicum* compared with classical staining procedures, taking into account that most of the species have similar karyotypes, with the majority of the chromosomes displaying small differences in length and arm ratio (cf. CHENNAVEERAIAH & HABIB 1966; LIMAYE & PATIL 1989; MOSCONE 1989, 1990, 1993; OHTA 1962; PICKERSGILL 1971, 1977, 1988, 1991).

The comparison of C-banded and classical karyotypes demonstrates good correspondence, but in addition, C-banding allows the identification of all homologous chromosome pairs within each taxon studied (with the exception of the sparsely banded chromosomes of *C. chacoënse*). However, it is still difficult to recognize homologous chromosome pairs between the different taxa.

Giemsa C-banding has revealed only few cases of heteromorphy between homologous chromosomes. This indicates a relatively low degree of structural heterozygosity, and suggests lack of hybridity as well as a low recombination rate for the taxa studied. These findings are in good agreement with the isozyme data obtained by JENSEN & al. (1979) and LOAIZA-FIGUEROA & al. (1989) in the genus, where self-compatibility is the rule, and where the cultivars in particular are predominantly self-fertile (cf. ESHBAUGH 1976; PICKERSGILL 1971, 1991).

The nuclear DNA content (BENNETT & SMITH 1976) and the total karyotype length (MOSCONE 1989) vary little in the *Capsicum* species studied so far, i.e., within a range of about 1 : 1.5, whereas the amount of constitutive heterochromatin displays a much greater variation, i.e., within a range of about 1 : 10. Among the taxa analysed here, there may be a weak correlation between the amounts of heterochromatin and nuclear DNA, but there is no obvious correlation between heterochromatin quantity and karyotype length. More detailed measurements of C-bands and nuclear DNA contents of other samples will be required to confirm these preliminary conclusions.

In respect to their C-banded karyotypes, significant differences exist between the taxa of *Capsicum* studied, both in the amount and the distribution of constitutive heterochromatin. On the basis of this karyological feature alone the species can be clearly separated from each other and also subdivided into two groups (I, II). Group I includes taxa with comparatively low C-heterochromatin content and consists of *C. chacoënse, C. parvifolium, C. annuum* var. *annuum*, and *C. baccatum* var. *pendulum*. In general, terminal bands are small and intercalary bands are completely absent or scarce in these species. Correspondingly, heterochromatic chromocentres in interphase nuclei are weakly represented. In this group, *C. chacoënse* has the lowest proportion of C-bands, about 4% of the total karyotype length. *C. parvifolium* and *C. annuum* var. *annuum* are rather similar, with about 5% and 7% of C-heterochromatin, respectively, but the former species lacks in-

tercalary bands. *C. baccatum* var. *pendulum* with about 12% exhibits the richest C-banding pattern within group I, occupying a position more separate from the other three species. At the same time, it is the closest to group II with a comparatively high C-heterochromatin content, which includes *C. pubescens* and *C. campylopodium*. Here, large blocks of telomeric bands are frequent in the karyotypes, and intercalary bands are also well represented. In relation to total karyotype length the two species exhibit C-bands of about 28% and 33%, respectively. Consequently, interphase nuclei show numerous large heterochromatic chromocentres. *C. pubescens* has large single bands in only one telomere of the longest pairs; on the other hand, *C. campylopodium*, the only species studied with 2n = 26, exhibits single or double bands (one terminal and another subterminal) in both telomeres of the longest pairs, the intercalary bands being more frequent among several chromosome pairs than in the former species.

These C-banding data correspond remarkably well with conclusions on systematic affinities and phylogenetic relationships in *Capsicum* based on crossing experiments and chromosome structures (PICKERSGILL 1988, 1991: in particular fig. 1), isozyme analyses (JENSEN & al. 1979; MCLEOD & al. 1982, 1983: in particular fig. 1; LOAIZA-FIGUEROA & al. 1989), flavonoid chemistry (BALLARD & al. 1970), morphology and phytogeography (e.g., HUNZIKER 1950, HEISER & SMITH 1953, ESHBAUGH 1983) and the classical karyological contributions already considered. A complete synthesis is hampered by the lack of C-banding data for several species (marked * in the following paragraphs).

Capsicum annuum and *C. baccatum* belong to the core of the long-recognized "white-flowered group" and to our low heterochromatin group I. Their hybrid progeny is viable, but their different isozyme patterns, chromosome structure and C-banding support their generally-recognized placement into two different subgroups, *C. annuum* together with *C. chinense* JACQ.*, *C. frutescens* L.*, and possibly also *C. galapagoënse* A. T. HUNZ.*, and *C. baccatum* together with the closely related (and sometimes included) *C. praetermissum* HEISER & SMITH* (having white or ± purplish flowers with greenish-yellowish spots).

Capsicum chacoënse is also a member of the "white-flowered group" and belongs to our low heterochromatin group I, exhibiting very little C-banding. It gives viable hybrid offspring with the *C. annuum* subgroup and also with *C. baccatum*, but differs so much in its chromosome structure, isozyme profile, and woody habit that it should be placed in a separate, probably ancestral *C. chacoënse* subgroup. This is also suggested by the isozyme data (JENSEN & al. 1979; MCLEOD & al. 1982, 1983), whereas a proposed intermediate position for this species between the "white-" and the "purple-flowered group" is not directly supported by our banding results.

Capsicum pubescens is a core species of the "purple-flowered group", well separated by crossing barriers, isoyzme data, and its high heterochromatin content (our group II). Close relatives are *C. eximium* A. T. HUNZ.* and *C. cardenasii* HEISER & SMITH*. A comparison of the C-banding pattern in *C. pubescens* and *C. baccatum* supports the suggestion from chromosome structure and isozyme analysis that the latter taxon could be a link between the *C. annuum* subgroup among the "white-" and the "purple-flowered group".

Also to be considered are two further woody and perennial x = 12 species with flower colour polymorphism (white to cream and purple individuals) which ap-

parently do not fit into the scheme of the two groups mentioned above: the widespread *C. parvifolium* and the local *C. tovarii* ESHBAUGH, SMITH & NICKRENT* from Peru. They could form a third and basal group in the genus. Chromosome banding data are still lacking for *C. tovarii*, but isozyme data (e.g., MCLEOD & al. 1983) and crossing experiments (e.g., PICKERSGILL 1991) document an isolated and approximately equidistant position between the "white-" and the "purple-flowered group" (genetic distance shortest from *C. chacoënse*, normal F_1 only with the *C. baccatum* subgroup). *C. parvifolium* belongs to our low heterochromatin group I with no intercalary bands, suggesting affinities with *C. chacoënse*, but nothing is yet known about its isozyme or crossing relationships.

The only taxa with x = 13 known so far within the genus are *C. campylopodium* (from SE Brazil; treated in this contribution), *C. rhomboideum* (HUMB. & BONPL.) KUNTZE* (the record apparently from Ecuador and cited as "*C. ciliatum*", identification by C. HEISER; should be reconfirmed), "*Capsicum* spec.* (southern Brazil)" (PICKERSGILL 1977, 1991: fig. 2 a), and several SE Brazilian accessions of *C. mirabile* MART.* (cited as "*C. pereirae*", "*C. friburguense*", and "*C. schottianum*"; MOSCONE 1989). Although PICKERSGILL (1977, 1991) hesitates about the inclusion of these x = 13 taxa in *Capsicum*, extensive taxonomic studies carried out by HUNZIKER (unpubl.) demonstrate their correct placement in this genus. The x = 13 taxa studied so far are characterized by more asymmetrical karyotypes [often including more than one (sub)telocentric pair!] and considerably more chromosome structural variation (MOSCONE 1989). A certain coherence of the x = 13 group is suggested, but Giemsa C-banding is available so far only for *C. campylopodium*. This banding pattern is rather similar to that of *C. pubescens*, a member of the "purple-flowered" x = 12 group, the two species forming our high heterochromatin group II, but there is no similarity of flower colour, as all the x = 13 taxa are white to yellowish. As long as no isozyme data or crossing experiments are available to support such relationships, the x = 13 taxa must be regarded as a separate but provisional taxonomic entity within *Capsicum* for which exomorphological features to characterize (or disassemble) it still have to be found.

In spite of the considerable variation of heterochromatin quantity among the species of *Capsicum*, a basic C-banding style, i.e., an aspect of generic quality, is maintained. Chromosomes always exhibit centromeric C-bands, even when they are small and difficult to see. Satellites are always strongly Giemsa-stained, the secondary constrictions remaining inconspicuous. The greatest variation concerns the telomeric bands (including satellites) which constitute the major proportion of total non-centromeric C-heterochromatin in all *Capsicum* taxa studied. In contrast, intercalary bands are often absent and represent only a minor C-heterochromatin component where they occur. In general, terminal and intercalary bands are unequally distributed over the two arms of the chromosomes and over the whole complement.

This C-banding style of *Capsicum* is quite comparable to those reported for other *Solanaceae*, e.g., for *Solanum tuberosum* L. and *S. phureja* JUZ. & BUK. (PIJNACKER & FERWERDA 1984) or for *Hyoscyamus muticus* L. (TYAGI & GILL 1990), but they have somewhat more asymmetrical chromosomes than most *Capsicum* taxa and a pronounced tendency for telomeric bands on the short arms. *Solanum stuckertii* BITT. (MOSCONE 1989), a link between *Solanum* and *Cyphom-*

andra s. str., which sometimes is placed in the latter genus, exhibits a reduction of centromeric bands (appearing in only two chromosome pairs). This trend becomes very obvious in *Cyphomandra* where centromeric bands are not visible, and where some pairs have no bands at all (PRINGLE 1990). Otherwise, *Cyphomandra* corresponds to the *Capsicum* pattern in respect to its strongly heterochromatic satellites, telomeric and less prominent intercalary bands of variable intensity, unequally distributed among the arms of the relatively symmetrical chromosome complements.

In contrast to the former *Solanoideae* genera, the C-banded karyotypes of *Cestrum* (BERG & GREILHUBER 1992 a, b) and *Nicotiana* (MOURAS 1982, MOURAS & al. 1986) from subfam. *Cestroideae*, have much less conspicuous telomeric bands in comparison to the often dominating clusters of intercalary bands. This is less obvious from the still fragmentary Giemsa C-data in *Petunia* (DIETRICH & al. 1981, WIJSMAN & al. 1983). Otherwise, proximal bands and heterochromatic satellites are present throughout these three genera.

Can the karyological data available for *Capsicum* help the understanding of the phylogeny and evolution of the genus? In respect to the chromosome base numbers: x = 12 and x = 13, there can be little doubt that x = 12, universal in the karyologically studied genera of *Solaneae* and dominant in *Capsicum* itself, corresponds to the ancestral condition as suggested by MOSCONE (1989). Thus, x = 13 in *C. campylopodium* and *C. mirabile* from SE Brazil, and in a population of the widespread *C. rhomboideum* from Ecuador, has to be regarded as a phylogenetic progression. In *C. campylopodium* x = 13 probably originates from x = 12 by centric (Robertsonian) fission of one long m chromosome (cf. MOSCONE, 1989). The two smallest asymmetrical chromosome pairs (12 and 13) of this species could have resulted in this way, with subsequent minor rearrangements. The banding pattern of these two short pairs suggests that the possible ancestral long m pair should have been symmetrically banded on both arms. Such chromosome pairs are actually found in *C. pubescens* (9 and 10) and in *C. campylopodium* itself (1, 2, and 4). From the similarities in the banding pattern of these two species, an origin of *C. campylopodium* with x = 13 from an ancestor with x = 12 and a karyotype comparable to *C. pubescens* appears likely. A parallel Robertsonian change has occurred in some cultivars of *Lycopersicon esculentum* MILL. They also exhibit the aberrant chromosome number 2n = 2x = 26, and apparently have also originated by centric fission (BANKS 1984). In respect to the whole assembly of SE Brazilian and Andean *Capsicum* taxa with x = 13 further studies will have to show whether they have a common (monophyletic) origin or not.

For interpreting the evolution of the x = 12 species of *Capsicum*, forming the core of the genus, our C-banding data are of obvious relevance. The universal presence of centromeric and satellite heterochromatin suggests that these are stable features and plesiomorphic ("ancient") components of the banding pattern (just as in *Asteraceae-Anthemideae*: SCHWEIZER & EHRENDORFER 1976). In contrast, the appearance and frequency of terminal and intercalary bands is variable, and hence, they should be regarded as apomorphic (and more "recent"). Consequently, the woody and perennial species, *C. chacoënse* and *C. parvifolium* from the Chaco and Catinga areas and beyond (Argentina, Paraguay, Bolivia, NE Brazil, Venezuela, and Colombia) in our low heterochromatin group I, with centromeric and satellite heterochromatic bands (but very few others) appear to be plesiomorphic and an-

cestral in this respect. Such an interpretation is supported by the basic position of *C. chacoënse* (and the possibly related *C. tovarii*) in respect to isozyme data dendrograms (JENSEN & al. 1979, MCLEOD & al. 1983) and the hypothesis that these species, together with *C. parvifolium* and their ancestors, could have been involved in the early evolution of the genus. On the other hand, the annual *C. pubescens* from the C and N Andes to C America (and *C. campylopodium* with x = 13) in our high heterochromatin group II, with an extensive C-banding pattern, should be regarded as most advanced. Finally, the subgroups with *C. annuum* and *C. baccatum* with wide, but Andean-centred distributions appear as connecting side lines. Such assumptions also agree with the isozyme data already cited.

It is interesting that in addition to *Capsicum* there are other groups of plants where an increase in constitutive heterochromatin and C-banding (particularly in terminal and intercalary bands) can be interpreted as an evolutionary advance (e.g., in *Anacyclus*: SCHWEIZER & EHRENDORFER 1976, EHRENDORFER & al. 1977; in the *Scilla bifolia* L. group: GREILHUBER 1979, GREILHUBER & al. 1981).

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