

Chromosome numbers in wild and semidomesticated Brazilian *Capsicum* L. (Solanaceae) species: do $x = 12$ and $x = 13$ represent two evolutionary lines?

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Chromosome numbers were determined in 29 accessions of wild and semidomesticated *Capsicum* species from the EMBRAPA Hortaliças (Brazil) germplasm collection. $2n = 24$ was found in *C. baccatum* var. *praetermissum*, *C. chinense*, *C. flexuosum* and *C. parvifolium*, while *C. buforum*, *C. campylopodium*, *C. cornutum*, *C. schottianum*, *C. villosum* var. *villosum* and five other native south-eastern Brazilian species not yet taxonomically named all had $2n = 26$ chromosomes. These are the first chromosome number determinations for *C. cornutum*, *C. schottianum*, *C. villosum* var. *villosum* and the five other Brazilian taxa. Our data confirm published data for *C. baccatum* var. *praetermissum*, *C. campylopodium*, *C. chinense*, *C. flexuosum* and *C. parvifolium*, but not for *C. buforum*. The prevalence of $2n = 26$ chromosomes among Brazilian species, along with the morphological and ecological characteristics presented by the wild and semidomesticated species occurring in Brazil, form a pattern different from that found in Andean wild and semidomesticated species. This supports the hypothesis that there are two different evolutionary lines in the genus and that the native south-eastern Brazilian species belong to the ancestral *Capsicum* gene pool. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, 151, 259–269.

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INTRODUCTION

Capsicum L. species, are native to the New World, but are cultivated widely in temperate and tropical regions, where they are used as vegetables, spices, ornamentals and for their medicinal properties. Most *Capsicum* species contain the alkaloid capsaicin, which determines their pungency, an important factor for the food and pharmaceutical industries.

Despite its importance and the volume of published data on many different aspects, knowledge about the origin and domestication of *Capsicum* is still contro-

versial. Archaeological evidence indicates that some species were already being cultivated in some parts of South and Central America between 7000 and 5000 BC. Peppers, together with *Phaseolus* L. and some Cucurbitaceae, are among the oldest cultivated plants in the Americas (Pickersgill, 1969).

The association of aspects such as the antiquity of the genus, its present broad geographical distribution and different ways and areas of cultivation, lead to a wide expression of genetic variability, as seen in the very different morphological types. This situation makes the establishment of taxonomic position and the determination of species relationships rather difficult tasks, and raises many questions that are still unanswered. Even so, there is a general consensus that the genus comprises 20–30 species, mostly classified as wild and semidomesticated, besides the five

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that are cultivated world-wide: *C. annuum* L. var. *annuum* (bell pepper), *C. baccatum* L. var. *pendulum* (Willd.) Eshbaugh (aji), *C. chinense* Jacq. (habanero), *C. frutescens* L. (tabasco) and *C. pubescens* Ruiz & Pavon (rocoto) (McLeod, Eshbaugh & Guttman, 1979a, 1979b; McLeod, Guttman & Eshbaugh, 1983a; McLeod, *et al.*, 1983b).

Brazil is considered to be the centre of diversity for the genus and has a richness of germplasm that is reflected in the large numbers of varieties cultivated all over the country, many of which are adapted to specific edapho-climatic microregions and have potential for genetic breeding (Buso *et al.*, 2001). Brazil also has the largest number of wild *Capsicum* species (Pickersgill, 1984; Bianchetti, 1996), although a comprehensive study of this diversity, especially of the native species, is still lacking.

The most frequent chromosome number in the genus, as well as in the Solanaceae as a whole, is $x = 12$ (Smith & Heiser, 1951; Lippert, Smith & Bergh, 1966; Pickersgill, 1971, 1977, 1991; Limaye & Patil, 1989; Moscone, 1992) and both the cultivated as well as the wild species with this basic number are 'true' *Capsicum* (Pickersgill, 1977, 1991). Until now, $x = 13$ has been found in *C. ciliatum* (H., B. & K.) Kuntze, *C. lanceolatum* (Green.) Morton & Stand. (Pickersgill, 1977, 1991; Tong & Bosland, 1997, 2003), *C. mirabile* Martius, *C. campylopodium* Sendtner. (Moscone *et al.*, 1993) and one unidentified south-eastern Brazilian species (Pickersgill, 1977). Natural polyploidy ($2n = 48$) has been reported so far for just one wild accession of *C. annuum* (Pickersgill, 1977). The occurrence of the two basic numbers $x = 12$ and 13 could suggest that there are two different evolutionary lines and that a taxonomic realignment of the genus might be necessary (Pickersgill, 1977; McLeod *et al.*, 1983b). For that, however, more comprehensive studies are needed and in particular a thorough examination of chromosome number distribution among wild *Capsicum* species, as well as the investigation of a possible relationship between chromosome numbers and morpho-ecological characteristics.

Therefore, in this paper we report chromosome numbers for a range of wild and semidomesticated *Capsicum* accessions from Brazil and discuss the data in relation to morpho-ecological information.

MATERIAL AND METHODS

All the accessions analysed are part of the *Capsicum* germplasm collection at EMBRAPA (Empresa Brasileira de Pesquisa em Agropecuária), Embrapa Hortaliças (CNPq), Brasília, DF, Brazil. The collections were made in several locations in the south-eastern region of Brazil, except for one accession from the Amazonian region (Table 1). Vouchers are kept at the

CENARGEN (Centro Nacional de Recursos Genéticos e Biotecnologia), EMBRAPA, Brasília, DF, Brazil.

Somatic chromosome numbers were determined in root-tip cells following the protocol of Pozzobon & Valls (1997) with minor modifications. A detailed karyotypic analysis was not performed, but overall chromosome size and shape, and presence of satellited chromosomes were recorded. Meiotic chromosome numbers were analysed in pollen mother cells from anthers fixed in 3 : 1 ethanol-acetic acid and squashed in 2% propionic carmine.

At least five (generally more than ten) cells with good chromosome spreading and no overlapping were analysed per plant. Semi-permanent slides were examined by light microscopy and the work recorded on photographs and with digital image capturing.

RESULTS AND DISCUSSION

CHROMOSOME NUMBER AND MORPHOLOGY

The chromosome numbers for 29 accessions of 2 semi-domesticated and 12 wild *Capsicum* species are listed in Table 1. These are the first determinations for *C. cornutum* (Hern.) A. T. Hunziker, *C. schottianum* Sendtner, *C. villosum* Sendtner var. *villosum* and the five new species. It can be seen that $2n = 26$ is the most common number among the wild species. No intraspecific variability for chromosome number was found and no apparent differences in the general size of chromosomes among species were detected. *Capsicum* chromosomes are about $3 \mu\text{m}$ in length.

For *C. baccatum* L. var. *praetermissum* (Heiser & Smith) A. T. Hunziker, the chromosome number $2n = 24$ confirms published data (Pickersgill, 1977; Bertão, 1993; Ferreira, 1998). This species has 11 pairs of metacentric chromosomes and one subterminal satellited pair (Fig. 1). The same result was found by Bertão (1993) and by Pickersgill (1977), who also reported intraspecific variation in the number and position of satellites.

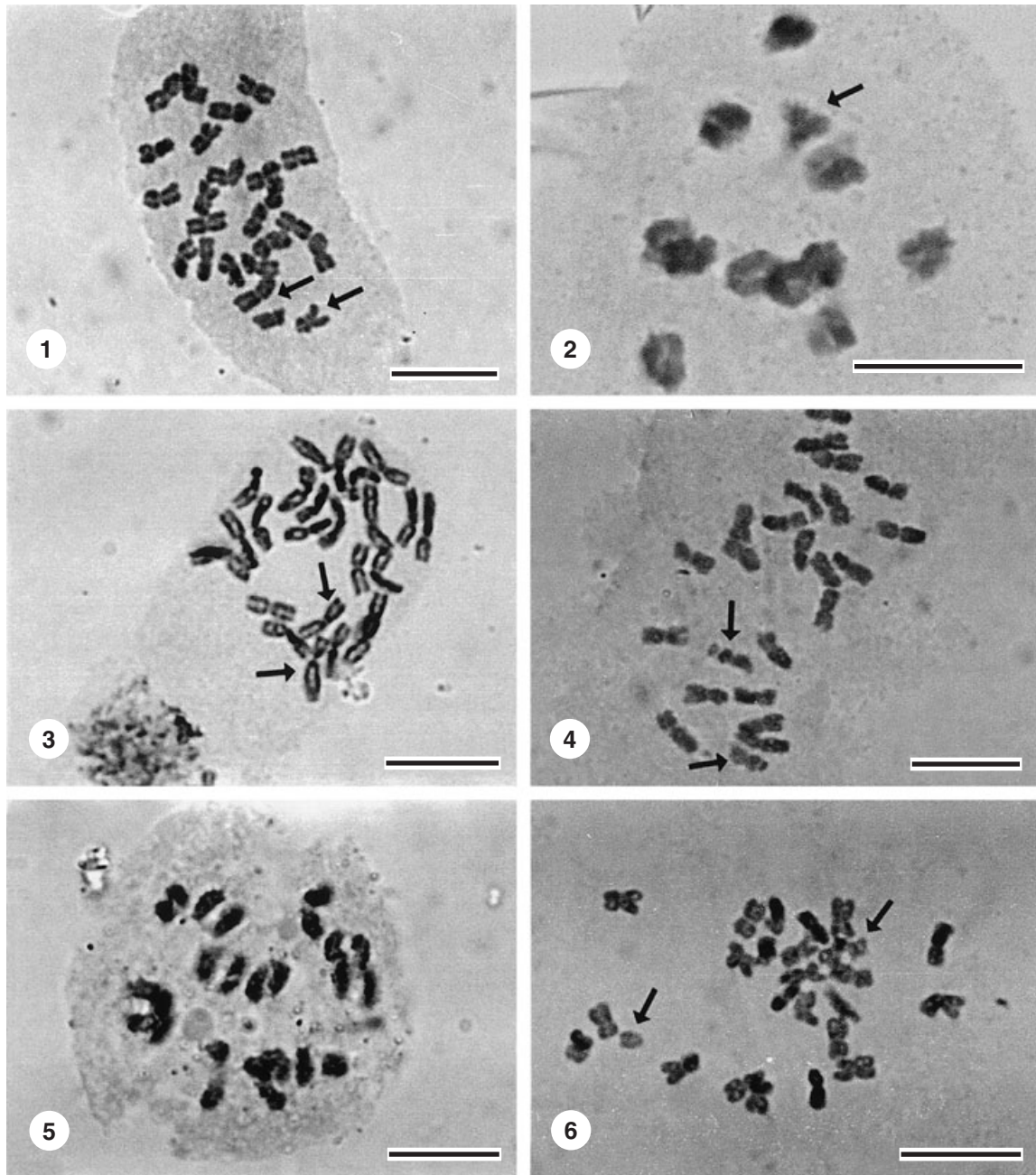
Capsicum chinense has $2n = 24$ chromosomes ($n = 12$, Fig. 2), the same number as found by several authors (Carluccio & Saccardo, 1977; Pickersgill, 1977; Limaye & Patil, 1989; Bertão, 1993; Moscone *et al.*, 1995, 2003; Moscone, Lambrou & Ehrendorfer, 1996; Ferreira, 1998), who do not concur on several aspects of karyotype morphology. However, as we have not analysed somatic cells, it is not possible to compare results on that aspect. The accession that we analysed is morphologically different from typical *C. chinense*, presenting characteristics of wild species and it has a twelfth chromosome pair smaller than in other $2n = 24$ species.

Among the wild species, only *C. flexuosum* Sendtner and *C. parvifolium* Sendtner had $2n = 24$, as

Table 1. *Capsicum* species and accessions examined

Species, CNPH ¹ accession number, collector's ² number	Place of collection ³	<i>n</i>	<i>2n</i>
Semi-domesticated			
<i>C. baccatum</i> var. <i>praetermissum</i>			
3303, LBB 1524	SP, Parque Estadual Jacupiranga	–	24
3325, LBB 1553	MG, Caldas	12	24
3326, LBB 1555	MG, Maria da Fé, Pedrão	–	24
<i>C. chinense</i>			
3727, LBB 1720	RR, Vila Apiaú, Roraima	12	–
Wild			
<i>C. flexuosum</i>			
3324, LBB 1552	MG, Caldas	12	24
<i>C. parvifolium</i>			
3331, LBB 1560	MG, University Federal de Viçosa	12	24
<i>C. buforum</i>			
3367, LBB 1550	SP, 1 km from Estação Eugene Lefèvre	13	–
3368, LBB 1554	MG, Maria da Fé, EPAMIG	13	–
<i>C. campylopodium</i>			
3335, LBB 1566	RJ, Santa Maria Madalena	13	26
<i>C. cornutum</i>			
3316, LBB 1542	SP, Estação Biológica da Boracéia	13	–
3320, LBB 1546	SP, Paraty-Cunha	13	26
3365, LBB 1527	SP, Parque Estadual Serra do Mar	13	–
<i>C. schottianum</i>			
3310, LBB 1535	SP, Estação Biológica da Boracéia	13	26
3311, LBB 1536	SP, Estação Biológica da Boracéia	13	26
3315, LBB 1540	SP, Estação Biológica da Boracéia	13	26
3318, LBB 1544	SP, Natividade da Serra-Bairro Alto SP	13	–
3319, LBB 1545	SP, São Luiz de Paraitinga	13	–
<i>C. villosum</i> var. <i>villosum</i>			
3313, LBB 1538	SP, Estação Biológica de Boracéia	13	26
3314, LBB 1539	SP, Estação Biológica da Boracéia	13	–
3317, LBB 1543	SP, Estação Biológica da Boracéia	–	26
3328, LBB 1557	RJ, Parque Nacional do Itatiaia	13	26
<i>Capsicum</i> sp. 1 ⁴			
3301, LBB 1521	SP, Parque Estadual Carlos Botelho	13	–
<i>Capsicum</i> sp. 2 (Oito dentes) ⁴			
3302, LBB 1523	SP, Parque Estadual Jacupiranga	13	26
3372, LBB 1525	SP, Estação Ecológica Juréia, Itatins	13	–
<i>Capsicum</i> sp. 6 (Piquete) ⁴			
3330, LBB 1559	MG, Lima Duarte	13	–
3337, LBB 1568	MG, Parque Nacional do Caparaó	13	–
3370, LBB 1564	RJ, Parque Nacional Serra dos Órgãos	13	–
<i>Capsicum</i> sp. 7 (Gruta do pião) ⁵			
3329, LBB 1558	MG, Parque Estadual do Ibitipoca	13	26
<i>Capsicum</i> sp. 8 (Flor lilás) ⁵			
3334, LBB 1565	RJ, Nova Friburgo	13	–

¹CNPH, Embrapa Hortaliças²LBB, Luciano de Bem Bianchetti³MG, Minas Gerais; SP, São Paulo; RJ, Rio de Janeiro; RR, Roraima⁴Putative new species, numbered and nicknamed following Bianchetti *et al.* (1999)⁵New species, being proposed as *C. pereirae* Hunz. and *C. friburguense* Hunz., Bianchetti & Barboza, respectively.



Figures 1–6. Somatic and meiotic chromosomes of *Capsicum* spp. Figs 1–4. *Capsicum* species with $2n = 24$ chromosomes. Fig. 1. Mitotic metaphase in *C. baccatum* var. *praetermissum*. Arrow points to a satellited subtelocentric. Fig. 2. Late diakinesis with 12 bivalents in *C. chinense*. Arrow points to the smallest pair of the complement. Fig. 3. Mitotic metaphase in *C. flexuosum*. Arrow points to a satellited submetacentric. Fig. 4. Mitotic metaphase in *C. parvifolium*. Arrow points to a satellited submetacentric. Figs 5, 6. Species with $2n = 26$ chromosomes. Fig. 5. Diakinesis in *Capsicum* sp. 1, with 13 bivalents. Fig. 6. Mitotic metaphase in *Capsicum* sp. 2. Arrow points to the small subtelocentric thirteenth pair. Scale bars = 10 μm .

found by Moscone (1992) and Bertão (1993) for *C. flexuosum* and by Bertão (1993) and Moscone (1993) for *C. parvifolium*. In both species we observed 11 metacentric pairs and one submetac-

entric satellited pair (Figs 3, 4). Bertão (1993) reported the same general chromosome morphology, but one subterminal satellited pair for *C. flexuosum*, while both Bertão (1993) and Moscone (1993) described 12

metacentric pairs, including the satellited one, for *C. parvifolium*.

These differences in morphology of the satellited chromosome pair between our results and other published ones, as well as among different authors for *C. chinense*, could reflect real biological differences or could be the consequence of technical differences such as pretreatment, chromosome condensation or even karyotype representation.

An interesting feature of one of the analysed accessions of *C. parvifolium* (3331) is that this accession was outstanding among the 363 accessions of the EMBRAPA Hortaliças *Capsicum* germplasm collection in its resistance to the fungus *Phytophthora capsici*, one of the most serious diseases of *Capsicum* in Brazil (Ribeiro *et al.*, 2003).

The eight accessions represented by two new (*Capsicum* sp. 7 and sp. 8) and three wild Brazilian species also assumed to be new all presented $2n = 26$ chromosomes. The thirteenth pair in these is a small one, less than half the size of the other 12 pairs (*c.* 1.4 μm), with an almost terminal centromere (Figs 5–8). Pickersgill (1977) also reported a small acrocentric thirteenth chromosome pair in one unidentified Brazilian species.

Capsicum buforum A. T. Hunziker (Figs 9, 10), *C. campylopodium* Sendtner (Fig. 11), *C. cornutum* (Figs 12, 13), *C. schottianum* (Figs 14–16) and *C. villosum* var. *villosum* (Figs 17, 18) also had $2n = 26$ chromosomes, the thirteenth being similar in size and shape to that of the new species. Tong & Bosland (2003) reported $2n = 24$ chromosomes in one accession of *C. buforum*. The characteristics that they reported for *C. buforum*, such as green mature fruits, three to four flowers per node and black seeds have been found so far in all (and only in) the $2n = 26$ species studied here. Therefore, a technical problem (e.g. sample misidentification) leading to an erroneous count by the above authors cannot be discounted. Moscone *et al.* (1993, 1995, 2003) also found $2n = 26$ in *C. campylopodium*, but with two small chromosome pairs. *Capsicum villosum* var. *villosum* was the only species analysed in which two satellited chromosome pairs could be identified, in accession 3317 (Fig. 17).

The origin of the small thirteenth pair in the $2n = 26$ species is still to be determined. Some rather speculative suggestions have been presented to date. The constancy in its number and behaviour during mitosis and meiosis supports Pickersgill (1977, 1991), who pointed out that they are not B chromosomes. Moscone *et al.* (1993) found similarities of C-banding patterns among the small pair and some regions of the other chromosomes in *C. campylopodium* (as well as in some chromosomes of the $2n = 24$ *C. pubescens*) and suggested that the small pair could have originated

through centric fission. In this case, a larger pair of chromosomes in the $2n = 26$ species with a reduced or absent short arm would be expected; however, based on published data and our own work, this type of chromosome has not been observed so far in *Capsicum* and therefore centric fission is an unlikely origin of the $2n = 26$ karyotype.

CHROMOSOME NUMBERS, MORPHO-ECOLOGICAL CHARACTERISTICS AND EVOLUTIONARY CONSIDERATIONS

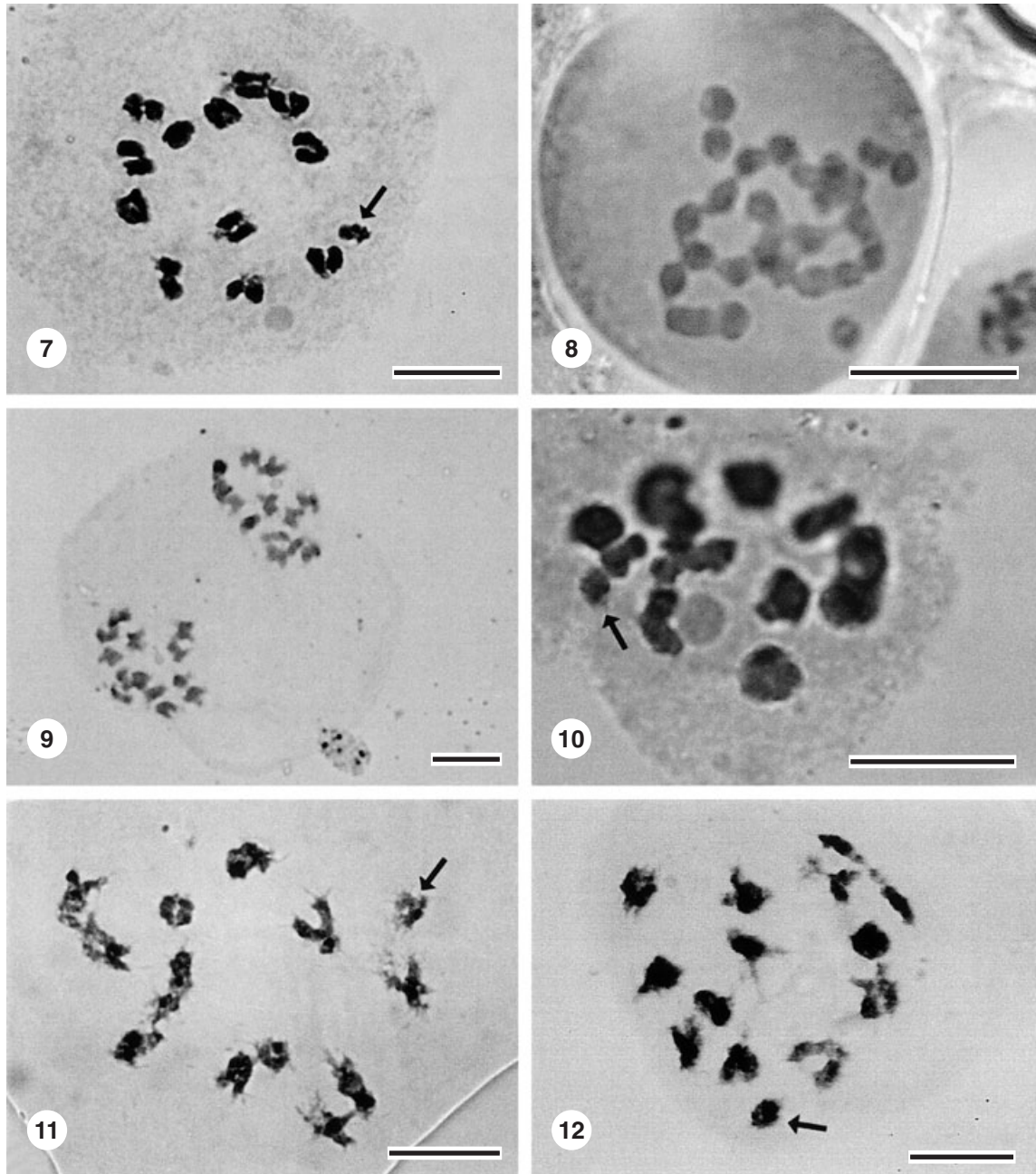
When the present results and published chromosome numbers for *Capsicum* species (Table 2) are compared, it can be seen that $2n = 24$ is the predominant chromosome number among the cultivated, semidomesticated and wild species (most of those from the Andean regions but also in *C. flexuosum* and *C. parvifolium*, which are native to Brazil). The four $2n = 24$ species that we analysed (the semidomesticated *C. baccatum* var. *praetermissum*, *C. chinense* and the wild *C. flexuosum* and *C. parvifolium*) share some morphological and ecological characteristics with most of the Andean wild species (all $2n = 24$).

According to Pickersgill (1969), wild *Capsicum* species have small, red, erect fruits and seeds are dispersed by birds attracted by the bright fruit colour. During domestication this kind of dispersal was lost, as man probably selected, consciously or not, non-deciduous pendant fruits. The change from erect to pendant fruits could have been due to the increase in fruit size and weight selected for in the domesticated forms. Such fruits, hidden among the leaves, would also be protected against predation by birds.

Capsicum baccatum var. *praetermissum* is endemic to the south-south-eastern region of Brazil (Bianchetti, 1996). Eventually, some plants might be found in other places, either wild or as escapees from cultivation. The species is found mostly as escapees in disturbed habitats or cultivated in small gardens. The fruits are deciduous, ovoid, rarely globular, bright red, erect and with pale yellow seeds. These morphological and ecological characteristics are shared with the wild Andean taxa.

Capsicum chinense is also found wild or under cultivation and is frequently used by the indigenous inhabitants of the northern Brazilian state of Roraima. Its fruits and seeds are morphologically similar to those of domesticated and wild Andean species (Barbosa *et al.*, 2002).

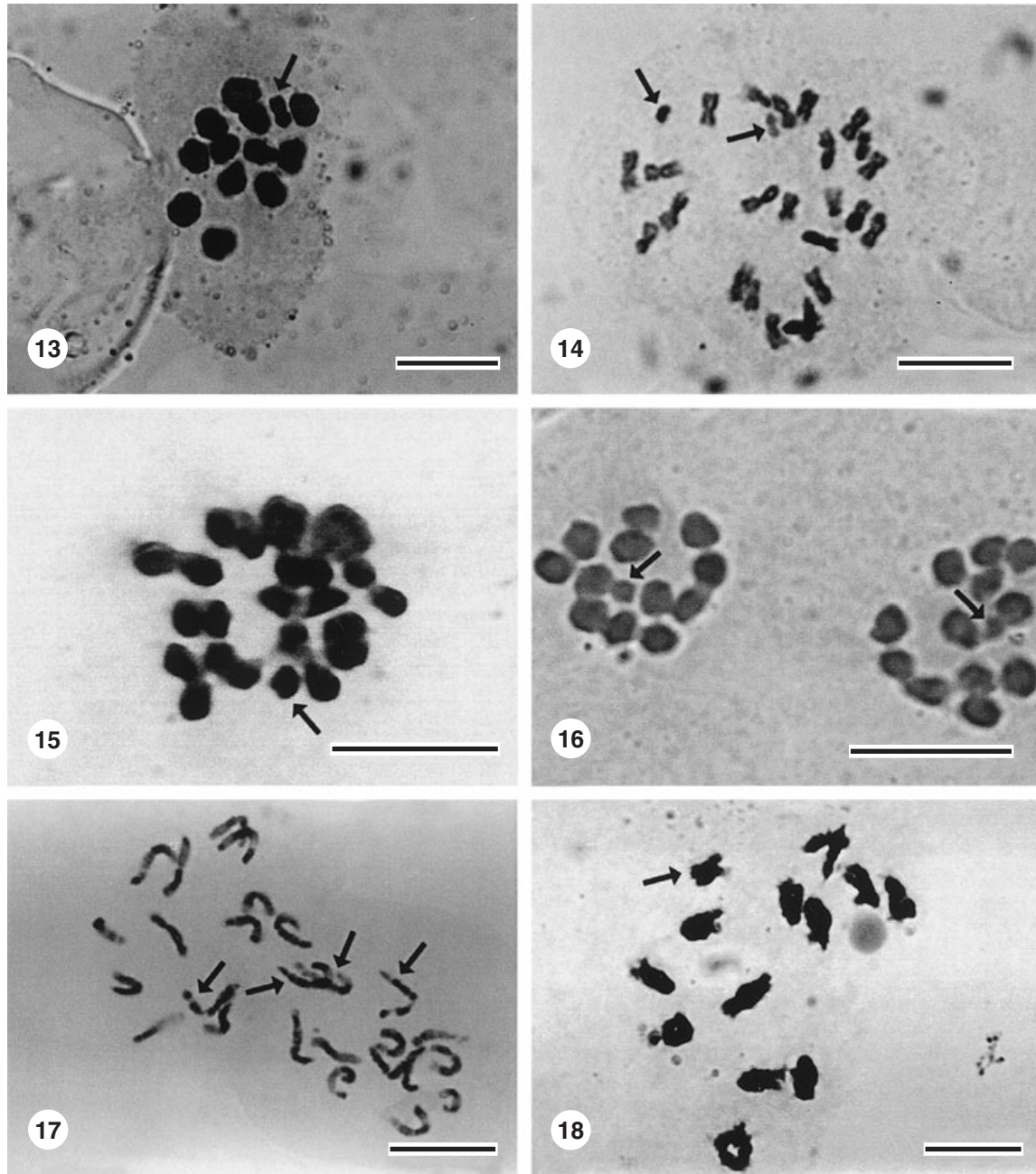
Capsicum flexuosum is normally distributed in low altitudes in a transition zone between the wild Andean species and those occurring in south-eastern Brazil, Paraguay and Argentina. It presents morphological characteristics from both groups of species: fruits are deciduous, globular, red or orange (charac-



Figures 7–12. Somatic and meiotic chromosomes of *Capsicum* species with $2n = 26$ chromosomes. Arrows point to the small thirteenth pair. Fig. 7. Diakinesis in *Capsicum* sp. 2, with 13 bivalents. Fig. 8. Anaphase I in *Capsicum* sp. 6, with sticky chromosomes. Fig. 9. Pro-metaphase II in *C. buforum* with a 13 + 13 chromosome distribution. Fig. 10. Diakinesis in *C. buforum*, with 13 bivalents. Fig. 11. Late diakinesis in *C. campylopodium*, with 13 bivalents. Fig. 12. Late diakinesis in *C. cornutum* with 13 bivalents. Scale bars = 10 μm .

teristic of Andean species adapted to dry climates), opaque and pendant, with blackish seeds (characteristic of Brazilian species adapted to wet climates). Bianchetti (1996) suggested that this mixed morphology could be explained by a recent evolutionary origin of *C. flexuosum*.

Capsicum parvifolium also presents morphological characteristics of both Andean and Brazilian species. The fruits are red or orange, with pale yellow seeds (characteristic of Andean species adapted to dry climates), globular and pendant (characteristic of Brazilian species adapted to wet climates). This is



Figures 13–18. Somatic and meiotic chromosomes of *Capsicum* species with $2n = 26$ chromosomes. Arrows point to the small thirteenth pair, except in Fig. 17. Fig. 13. Metaphase I in *C. cornutum* with 13 bivalents. Fig. 14. Mitotic metaphase in *C. schottianum*. Fig. 15. Metaphase I in *C. schottianum* with 13 bivalents. Fig. 16. Anaphase I in *C. schottianum* with 13 + 13 chromosome distribution. Fig. 17. Mitotic metaphase in *C. villosum* var. *villosum* with two pairs of satellited chromosomes (arrows). Fig. 18. Late diakinesis in *C. villosum* var. *villosum* with 13 bivalents. Scale bars = 10 μm .

the only species with a disjunct geographical distribution in dry areas in Venezuela, Colombia and north-eastern Brazil (Bianchetti, 1996). According to Bianchetti (1996), this present distribution could be explained by the following scenarios: a very wide distribution of the species during the late glacial

period dwindled, owing to the enlarging Argentinian – Paraguayan – Bolivian arid region, leaving open vegetation areas which were later isolated; or a humid tropical region expanded over South America, including north-eastern Brazil, and a later retreat of the forest and consequent expansion of

Table 2. Chromosome numbers reported for *Capsicum* species

Species	2n	References
Domesticated		
<i>C. angulosum</i> Mill.	24	Fedorov (1969); syn. to <i>C. baccatum</i> L. ¹
<i>C. annuum</i> L.	24	Fedorov (1969); Moore (1973); Pickersgill (1977); Goldblatt (1981, 1984, 1985, 1988); Goldblatt & Johnson (1990, 1991, 1994, 1996, 1998, 2000); Bertão (1993); Ferreira (1998)
	48	Pickersgill (1977)
<i>C. baccatum</i> L.	24	Moore (1973); Pickersgill (1977) Goldblatt (1981); Bertão (1993); Goldblatt & Johnson (1991, 1996); Ferreira (1998)
<i>C. baccatum</i> L. var. <i>pendulum</i> (Willd.) Eshbaugh	24	Goldblatt & Johnson (1990, 1998, 2000); Moscone <i>et al.</i> (1993, 2003)
<i>C. baccatum</i> L. var. <i>umbilicatum</i> (Vellozo) Hunz. & Barboza	24	Moscone (1999); Moscone <i>et al.</i> (2003); syn. to <i>C. baccatum</i> L. ¹
<i>C. chinense</i> Jacq.	24	Pickersgill (1977); Limaye & Patil (1989) Bertão (1993); Goldblatt & Johnson (1990, 1994); Ferreira (1998); Moscone <i>et al.</i> (1995, 1996, 2003); this work.
<i>C. cordiforme</i> Mill.	24	Fedorov (1969); syn. to <i>C. annuum</i> var. <i>annuum</i> ¹
<i>C. frutescens</i> L.	24	Fedorov (1969); Moore (1973); Pickersgill (1977); Goldblatt (1981, 1988); Bertão (1993); Goldblatt & Johnson (1991, 1996, 1998, 2000); Moscone <i>et al.</i> (2003)
<i>C. pendulum</i> Willd.	24	Fedorov (1969); Goldblatt & Johnson (1991); syn. to <i>C. baccatum</i> var. <i>pendulum</i> ¹
<i>C. pubescens</i> Ruiz & Pavon	24	Fedorov (1969); Pickersgill (1977); Goldblatt & Johnson (1991, 1994, 1998, 2000); Moscone <i>et al.</i> (2003)
<i>C. sinense</i> Murr.	24	Fedorov (1969); probably <i>C. chinense</i> Jacq. ²
Semi-domesticated		
<i>C. baccatum</i> L. var. <i>baccatum</i> Eshbaugh	24	Goldblatt & Johnson (1990); Moscone <i>et al.</i> (2003)
<i>C. baccatum</i> var. <i>praetermissum</i> (Heiser & Smith) Hunz.	24	Fedorov (1969); Pickersgill (1977); Bertão (1993); Ferreira (1998); this work; syn. to <i>C. praetermissum</i> ¹
<i>C. cardenasii</i> Heiser & Smith	24	Fedorov (1969), Pickersgill (1977)
<i>C. chacoense</i> Hunz.	24	Fedorov (1969); Pickersgill (1977); Bertão (1993); Goldblatt & Johnson (1991, 1994, 1996, 1998); Moscone <i>et al.</i> (2003)
<i>C. eximium</i> Hunz.	24	Fedorov (1969); Pickersgill (1977); Moscone <i>et al.</i> (2003)
<i>C. microcarpum</i> Cav.	24	Fedorov (1969); Goldblatt & Johnson (1991); syn. to <i>C. baccatum</i> ¹ (probably var. <i>baccatum</i> ²)
<i>C. tovarii</i> Eshbaugh, P. G. Sm. & Nickrent Wild	24	Goldblatt (1985)
<i>C. buforum</i> Hunz.	24	Tong & Bosland (2003)
	26	This work
<i>C. campylopodium</i> Sendt.	26	Moscone <i>et al.</i> (1993, 1995, 2003), this work
<i>C. ciliatum</i> (H., B. & K.) Kuntze	26	Pickersgill (1977)
<i>C. cornutum</i> (Hern.) Hunz.	26	This work
<i>C. flexuosum</i> Sendt.	24	Moscone (1992), Bertão (1993), this work
<i>C. galapagense</i> Heiser & Smith	24	Fedorov (1969); Pickersgill (1977)
<i>C. lanceolatum</i> (Green.) Morton & Standl.	26	Tong & Bosland (1997, 2003)
<i>C. macrophyllum</i> Standl.	24	Fedorov (1969); re-classified as <i>Witheringia solanacea</i> L'Her. ¹
<i>C. maculatum</i> Standl. & Mort.	24	Fedorov (1969); re-classified as <i>Witheringia maculata</i> (Morton & Standl.) Hunz. ¹
<i>C. mirabile</i> Mart. var. <i>mirabile</i>	26	Moscone <i>et al.</i> (1995)
<i>C. parvifolium</i> Sendt.	24	Bertão (1993); Moscone (1993); Moscone <i>et al.</i> (1993, 1995, 2003); this work
<i>C. schottianum</i> Sendt.	26	This work
<i>C. stenophyllum</i> Mort. & Standl.	24	Fedorov (1969); re-classified as <i>Witheringia meiantha</i> (Don. Sm.) Hunz. ¹
<i>C. stramonifolium</i> (H., B. & K.) Standl.	24	Fedorov (1969); re-classified as <i>Witheringia stramonifolia</i> Kunth ¹

Table 2. *Continued*

Species	2n	References
<i>C. testiculatum</i> Vis. ex Dun.	24	Goldblatt & Johnson (1991); not considered as related to <i>Capsicum</i> ¹
<i>C. tetramerum</i> Stand. & Mort.	24	Fedorov (1969); re-classified as <i>Witheringia solanacea</i> var. <i>solanacea</i> LHer. ¹
<i>C. villosum</i> Sendt. var. <i>villosum</i>	26	This work
<i>Capsicum</i> sp. (Brazil)	26	Pickersgill (1977)
<i>Capsicum</i> sp. 1 ³	26	This work
<i>Capsicum</i> sp. 2 ³	26	This work
<i>Capsicum</i> sp. 6 ³	26	This work
<i>Capsicum</i> sp. 7 ³	26	This work
<i>Capsicum</i> sp. 8 ³	26	This work

¹According to Baral & Bosland (2002)

²L. B. Bianchetti (pers. comm.)

³Bianchetti *et al.* (1999)

open vegetation formed refugia in which *C. parvifolium* is found.

It should be noted that fruit colour in these four species is similar to that of the Andean species, but the pendant fruits of *C. flexuosum* and *C. parvifolium* are a characteristic shared by most Brazilian taxa. Both species have undergone environmental changes and some morphological variation, but have maintained the chromosome number of $2n = 24$.

The other wild species analysed present a set of morphological and ecological discriminatory characteristics, including: (1) a more coastal (eastern) distribution, usually above 400 m a.s.l.; (2) occurrence in humid forests; (3) fruits that are globular, pendant and greenish-yellowish when ripe with blackish seeds, suggesting that the natural pollinators are probably not birds (Bianchetti, 1996; Bianchetti *et al.*, 1999). We have showed that another distinctive characteristic of these species is the chromosome number of $2n = 26$, with the thirteenth pair morphologically different from the other chromosomes of the complement. The joint analysis of these morpho-ecological and cytological aspects strongly suggests the existence of two distinct evolutionary lines. However, how the difference in chromosome numbers is related to phylogenetic relationships is still not clear.

A few other species from the Andean or north-western South American regions, such as *C. mirabile* and an unidentified species, also have $2n = 26$ chromosomes (Pickersgill, 1977, 1991; Moscone *et al.*, 1993; Tong & Bosland, 1997, 2003). Tong & Bosland (2003) attempted interspecific crosses between the $2n = 26$ *C. lanceolatum* from Guatemala and several $2n = 24$ species [*C. pubescens*, *C. praetermissum*, *C. ciliatum* (H., B. & K.) Kuntze, *C. baccatum*, *C. frutescens*, *C. buforum*, *C. cardenasii* Heiser & Smith, *C. eximium*

Hunz. and *C. tovarii* Eshbaugh] and verified the existence of incompatibility between the $2n = 26$ species and the other taxa.

Data in Table 2 show that $x = 13$ is more common among the wild Brazilian species than in the other *Capsicum* taxa studied so far. Is the $x = 13$ a primitive or a derived characteristic? Have the $x = 13$ species originated from a $x = 12$ ancestor and acquired one pair of chromosomes (the small pair); have the $x = 12$ species originated from the $x = 13$ ones, or have both group of species originated from a common ancestor, having afterwards undergone distinct evolutionary trends? Bianchetti (1996), examining morpho-ecological characteristics of these $x = 13$ species, suggested that they represent a primitive condition. Moreover, the occurrence of the $x = 13$ species, *C. lanceolatum* in Guatemala and *C. ciliatum* in north-western South America, could represent the remains of an ancient continuous vegetation linking the eastern South American region (where the Brazilian wild $x = 13$ species are) and the north-western region (Bianchetti, 1996).

Based on his own data and those of Landrum (1981) on *Myrceugenia* (Myrtaceae), a genus with distribution and phylogenetic problems similar to those of *Capsicum*, Bianchetti (1996) formulated the hypothesis that the genus *Capsicum* was distributed in a continuous humid forest vegetation belt extending from the Brazilian south-east to near to Chile. These species, with $x = 13$, had pendant, greenish-yellowish fruits that were disseminated by forest-inhabiting vectors, probably bats. With the elevation of the Andes, the area located to the east (Argentina) became semiarid and there followed an extreme reduction of the forest, leading to a selection pressure favouring species with the potential to adapt to the new environment. In some of the populations one pair of

chromosomes was lost and the surviving $x = 12$ taxa changed fruit position and colour to erect and reddish and the dispersal vectors changed to open-vegetation inhabitants, probably birds. Afterwards, these species migrated northwards through the Andes to regions of intermediate altitudes and mild climates. In south-eastern Brazil, the vegetation was little altered owing to the region's climatic stability, so the species from that area maintained the primitive characteristics of pendant greenish-yellowish fruits.

CONCLUDING REMARKS

Existing data suggest a clear separation between the wild Brazilian *Capsicum* species with $x = 13$ and the others, mostly with $x = 12$. Morpho-ecological data (Bianchetti, 1996) suggest that Brazilian species are primitive and the others are derived. Cytogenetic information obtained so far cannot confirm whether $x = 13$ is primitive or derived, but a comparison of our cytological work and the morpho-ecological data suggests that $x = 13$ is the ancestral basic number and that the two basic chromosome numbers represent two different evolutionary lines. Further studies in a larger number of species and populations, including FISH (Fluorescent *in situ* Hybridization) analysis, use of DNA markers and crossing experiments, are necessary to elucidate the evolutionary history of *Capsicum*.

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