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Karyology and cytotaxonomy of the genus *Passiflora* L. (Passifloraceae)

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Abstract. The chromosomes of 31 species of *Passiflora*, distributed throughout the subgenera *Astrophea*, *Calopathanthus*, *Distephana*, *Dysosmia*, *Passiflora*, *Plectostemma* and *Tacsonia* were analysed. Three different karyotypes were observed: 2n = 12, 24, 36; 2n = 18, 72 and 2n = 20. The karyotype of these species was almost always constituted of metacentric and submetacentric chromosomes with variable karyotype symmetry. In the group with x = 6, represented by the subgenus *Plectostemma*, six diploid species with 2n = 12, one tetraploid with 2n = 24 (*P. suberosa*) and an intraspecific polyploid with 2n = 12, 36 (*P. misera*) were analysed. *P. pentagona* (subgenus *Astrophea*) may also be included in this karyological group since it presents 2n = 24 and may be of polyploid origin, with x = 6. The interphase nuclei in this group were areticulate, except those of *P. morifolia* and *P. pentagona* with semi-reticulate characteristics. Two small terminal heterochromatic blocks, positive for chromomycin A_3, were identified in the largest chromosome pair of *P. capsularis* and *P. rubra*, species very closely related, while *P. tricuspis* displayed four chromosomes with proximal blocks. In the group with x = 9, represented mainly by subgenus *Passiflora*, 20 species with 2n = 18 and one with 2n = 72 were studied. They presented chromosomes larger than those species with x = 6 and interphase nuclei of semi-reticulate type, except for *P. mixta* with areticulate nuclei. Four terminal CMA^+ blocks were observed in *P. edulis*, six blocks in *P. caerulea* and *P. racemosa*, while five blocks were observed in the single *P. amethystina* plant analysed. *P. foetida* (subgenus *Dysosmia*), the only species with 2n = 20, exhibited six chromosomes with CMA^+ blocks and interphase nuclei of the areticulate type. The meiotic analysis of representatives of the three groups (*P. foetida*, *P. suberosa*, *P. cincinnata* and *P. racemosa*) always presented regular pairing and regular chromosome segregation, except in *P. jilekii* where a tetravalent was observed. The analysis of the chromosome variation within the genus and the family suggests that the base number of *Passiflora* may be $x_1 = 6$ or $x_1 = 12$, whereas $x_2 = 9$ is only an important secondary base number.

Key words: Cytotaxonomy, *Passiflora*, fluorochrome staining, mitotic chromosomes.

The genus *Passiflora* consists of a group of herbaceous or woody vines, usually climbing by tendrils, rarely erect herbs, small trees or shrubs, typically tropical and of American origin (Killip 1938). The systematics of the genus appears little defined as yet, and the number of species currently known is around 465 (Vanderplank 1996). Many *Passiflora* are cultivated as ornamentals or for their edible

fruits or medicinal properties (Manica 1981, Martin and Nakasone 1970).

Cytologically, *Passiflora*, as other genera of Passifloraceae, have been poorly studied. Prior to this date, the chromosome numbers of 75 species were known, representing about 16.1% of the genus. The most frequent chromosome numbers were n = 6 and n = 9, but n = 7, 10, 11, 12, 18 and 42 have also been registered. This numerical variation gave great support to the infrageneric taxa proposed by Killip (1938). Several authors have proposed different base numbers (x = 3, x = 6 and x = 9) for the genus, without a clear understanding of this variation and the phylogenetic relationships between the species (see Storey 1950, Raven 1975, Morawetz 1986). In the present work, 31 species of *Passiflora* were cytologically analysed by conventional staining, seeking to identify the basic chromosome number of the genus and the relationships between different haploid numbers. In addition, seven species were investigated with the fluorochromes chromomycin-A_3 (CMA) and 4′,6-diamidino-2-phenylindole (DAPI), which bind preferentially to GC-rich or AT-rich DNA, respectively, allowing the localisation of particular types of heterochromatin in different karyotypes (Schweizer 1976, Deumling and Greilhuber 1982).

Material and methods

Most of the material was collected either on field trips in Brazil or obtained from other institutions. Voucher specimens of the materials collected at the Royal Botanic Gardens, Kew, are deposited in the herbarium of this institution whereas all others are at the UFP herbarium, in the Federal University of Pernambuco. The species analysed, their chromosome numbers, voucher numbers and provenance are listed in Table 1.

For cytogenetic study, young root tips were collected from adult plants or obtained from seeds germinated in Petri dishes. Root tips were pretreated with 0.002M 8-hydroxyquinoline at 4 °C for 24 hours, fixed in ethanol-acetic acid (3:1) overnight at room temperature and stored at −20 °C. Floral buds were fixed directly in ethanol-acetic acid (3:1) for meiotic analysis. Conventional chromosome staining with Giemsa proceeded as described by Guerra (1983). Staining with fluorochromes CMA and DAPI was carried out as described by Deumling and Greilhuber (1982). In some species the chromosome sizes of one to four metaphases were estimated from amplified negative images using a micrometric scale of the same enlargement. Photomicrographs were taken with Agfa Copex Pan ASA 25 film, for conventional staining, and Kodak Tri-X Pan ASA 400, for fluorescent staining. We followed the taxonomical system by Killip (1938).

Results

The chromosome numbers observed (Table 1) divide the species studied into three groups: nine species with 2n = 12, 24 and 36; 21 species with 2n = 18, 72; one species with 2n = 20.

In the group of species with 2n = 12, 24, 36, almost all were diploid with 2n = 12, except *P. suberosa* L. with 2n = 24. *P. pentagona* Mast. (2n = 24) may also be included in this group since it is karyologically very similar. However, it belongs to a different subgenus [subgenus *Astrophea* (DC.) Mast.] and may be not related to the 2n = 24 species of this group. *P. misera* Kunth presented intraspecific polyploidy with 2n = 12 and 2n = 36 (Fig. 1a–e). In the root meristem of *P. morifolia* Mast. tetraploid cells (2n = 24) were often found together with normal diploid ones (Fig. 1g, h).

The chromosome morphology in species of this first group varied between metacentric and submetacentric. The karyotype was more symmetrical in *P. herbertiana* Ker Gawl. and *P. morifolia*, with small and gradual size variation among the chromosomes. In the other diploid species the karyotype was asymmetric, with one or two larger metacentric pairs. In the tetraploid *P. suberosa* the karyotype was also asymmetric. The haploid complement size estimated in *P. morifolia*, *P. tricuspis* and in the diploid *P. misera* was 13.43, 10.89 and 9.93 μm, respectively, and the ratio of the largest over the smallest chromosome pairs was 1.72, 1.67 and 1.87 μm, respectively.

Table 1. List of the *Passiflora* species analysed with respective chromosome numbers, herbarium vouchers and provenances

Taxon	n	2n	Voucher number	Provenance
Subgenus *Astrophea* (DC.) Mast.				
Section *Pseudoastrophea* (Harms) Killip				
P. pentagona Mast.	12	–	9097	– Palmeiras, BA, Brazil
Subgenus *Plectostemma* Mast.				
Section *Cieca* (Medic.) Mast.				
P. coriacea Juss.	–	12	PAS-448	– Botanical Garden of Vienna, Austria
P. morifolia Mast.	–	12	PAS-251	– Botanical Garden of Vienna, Austria
P. suberosa L.	12	24	PAS-245	– Botanical Garden of Vienna, Austria
Section *Decaloba* (DC.) Mast.				
P. herbertiana Ker Gawl.	–	12	PAS-465	– Royal Botanic Gardens, Kew, England
P. misera Kunth	–	12	PAS-211	– Parque do Turvo, RS, Brazil
	–	36	PAS-095	– Dois Irmãos, Recife, PE, Brazil
P. tricuspis Mast.	–	12	PAS-1532	– Bosque Municipal, São José do Rio Preto, SP, Brazil
Section *Xerogona* (Raf.) Killip				
P. capsularis L.	–	12	PAS-453	– Botanical Garden of Vienna, Austria
P. rubra L.	–	12	PAS-351	– Mata do Saltinho, Rio Formoso, PE, Brazil
Subgenus *Tacsonia* (Juss.) Triana & Planc.				
P. mixta L.	–	18	PAS-459	– Royal Botanic Gardens, Kew, England
Subgenus *Distephana* (Juss.) Killip				
P. coccinea Aubl.	–	18	PAS-469	– Botanical Garden of Vienna, Austria
P. glandulosa Cav.	–	18	PAS-242	– Natal, RN, Brazil
Subgenus *Calopathanthus* (Harms) Killip				
P. racemosa Brot.	9	–	PAS-456	– Botanical Garden of Vienna, Austria
Subgenus *Passiflora*				
Serie *Quadrangularis* (Harms) Killip				
P. alata Curtis	–	18	PAS-236	– Mata do Saltinho, Rio Formoso, PE, Brazil
P. quadrangularis L.	–	18	PAS-452	– Botanical Garden of Vienna, Austria
P. x *allardii* (*P. caerulea* x *P. quadrangularis*)	–	18	PAS-466	– Royal Botanic Gardens, Kew, England
Serie *Laurifoliae* Killip ex Cervi				
P. nitida Kunth	–	18	PAS-457	– Royal Botanic Gardens, Kew, England
Serie *Setaceae* Killip ex Cervi				
P. setacea DC.	9	–	9090	– Utinga, MG, Brazil

Table 1 (continued)

Taxon	n	2n	Voucher number	Provenance
Serie *Passiflora*				
P. cincinnata Mast.	9	–	PAS-227	– Natal, RN, Brazil
P. edulis Sims. f. *flavicarpa* Deg.	–	18	PAS-016	– Cultivated, Recife, PE, Brazil
	–	18	PAS-1568	– Cultivated, Recife, PE, Brazil
Serie *Kermesinae* Killip ex Cervi				
P. edmundoi Sacco	–	18	9101	– Palmeiras, BA, Brazil
P. kermesina Link & Otto	–	18	PAS-217	– Recife, PE, Brazil
	–	18	PAS-226	– Natal, RN, Brazil
Serie *Simplicifoliae* (Harms) Killip				
P. actinia Hook.	–	18	PAS-909	– Curitiba, PR, Brazil
P. galbana Mast.	–	18	9381	– Camocim de São Félix, PE, Brazil
P. jilekii Wawra	9	–	9331	– Caparaó, ES, Brazil.
P. mucronata Lam.	–	18	PAS-098	– Serrambi, Ipojuca, PE, Brazil
	–	18	PAS-218	– Itamaracá, PE, Brazil.
	–	18	PAS-297	– N. S. da Ajuda, BA, Brazil
Serie *Lobatae* (Harms) Killip				
P. amethystina Mikan var. *amethystina*	–	18	PAS-630	– Rio do Corvo, Quatro Barras, PR, Brazil
	–	18	9334	– Domingos Martins, ES, Brazil
P. caerulea L.	–	18	PAS-449	– Botanical Garden of Vienna, Austria
P. subpeltata Ortega	–	18	PAS-463	– Royal Botanic Gardens, Kew, England
Serie Unknown				
Passiflora sp.	–	72	PAS-206	– Mata do Saltinho, Rio Formoso, PE, Brazil
Subgenus *Dysosmia* (DC.) Killip				
P. foetida L.	–	20	PAS-190	– Campus/UFPE, Recife, PE, Brazil
	–	20	PAS-193	– Dois Irmãos, Recife, PE, Brazil
	10	–	PAS-214	– Ponte dos Carvalhos, Cabo de Sto Agostinho, PE, Brazil
	–	20	PAS-243	– São Luís, MA, Brazil
	–	20	PAS-1092	– Cabo de Sto. Agostinho, PE, Brazil
	–	20	PAS-1553	– Petrolina, PE, Brazil

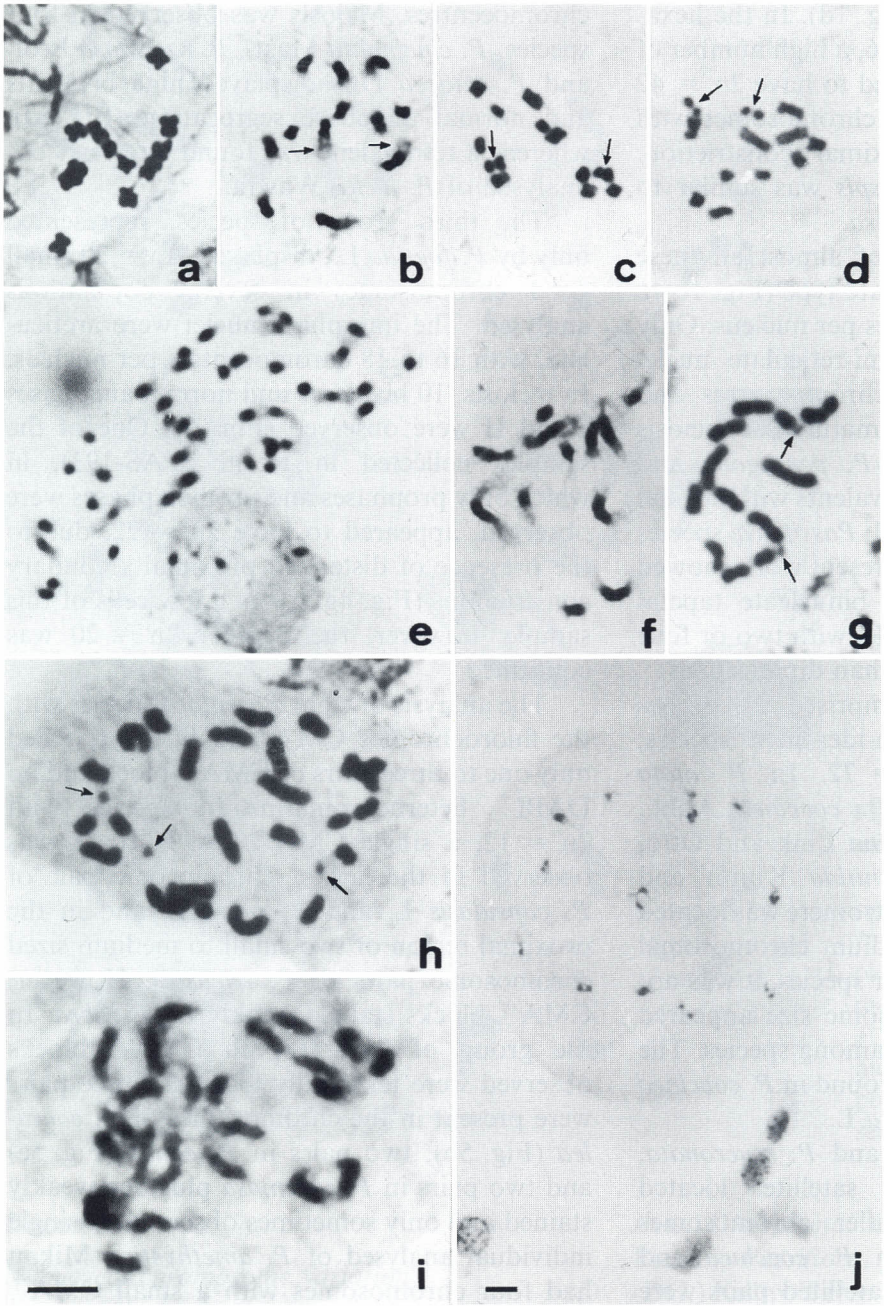

Fig. 1. Chromosome complements of *Passiflora* species with x = 6. **a** *P. herbertiana* (2n = 12); **b** *P. capsularis*; **c** *P. tricuspis* (2n = 12); **d** *P. misera* (2n = 12); **e** *P. misera* (2n = 36); **f** *P. coriacea* (2n = 12); **g** *P. morifolia* (2n = 12); **h** Tetraploid root tip metaphase of *P. morifolia* (2n = 24); **i** Diakinesis of *P. pentagona* (n = 12); **j** Diakinesis of *P. suberosa* (n = 12). Note the areticulate interphase nuclei in **e** and **j**. Arrows point out secondary constrictions in **b**, **c** and **d** and satellites in **g** and **h**. All figures are in the same magnification, except **j**. Bars in **i** and **j** represent 5 μm

Satellites or secondary constrictions were generally observed in one of the smaller chromosome pairs of *P. morifolia*, *P. tricuspis* Mast. and in the diploid *P. misera*. The secondary constriction was proximal in the latter two and sub-terminal in the former (Fig. 1c, d–g). The terminal regions of prophase and prometaphase chromosomes were characteristically less condensed, especially in the longer pairs. This was more evident in the largest pair of diploid *P. misera* (Fig. 1d) and in the three largest pairs of its hexaploid (Fig. 1e).

P. misera with 2n = 12 presented chromosomes of clearly differentiated sizes. The largest pair was almost twice the length of the smallest one. One of the shorter pairs presented a secondary proximal constriction, which when distended suggests an erroneous count of

2n = 14 chromosomes (Fig. 1d). In the hexaploid cytotype with 2n = 36, a high number of prometaphase cells appeared to have 2n = 42 due to the presence of six chromosomes with distended secondary proximal constriction. The karyotype of *P. tricuspis* was similar to the diploid form of *P. misera*.

The interphase nuclei of almost all these species were of the areticulate type (Fig. 1e–j), with 10 to 12 chromocentres per nucleus. Only *P. morifolia* presented semi-reticulate nuclei with six to eight small chromocentres and deeper stained diffuse chromatin. The meiosis of two species analysed, *P. pentagona* and *P. suberosa*, displayed 12 bivalents with normal segregation (Fig. 1i, j). All *Passiflora* species analysed in meiosis in the present work showed very large meiocytes and binucleate tapetal cells bearing polyploid nuclei with two or four times more chromosomes than diploid ones.

The second group comprised 21 species with 2n = 18 and a non-identified species, *Passiflora* sp., with 2n = 72. In *P. alata* Curtis, *P. caerulea* L., *P. coccinea* Aubl., *P. edulis* Sims., *P. kermesina* Link and Otto, *P. mucronata* Lam., *P. nitida* Kunth and *P. subpeltata* Ortega the centromere was located in the medium or sub-medium chromosomal region, whereas in the other species, it was not clearly observed. Chromosome size appeared to be quite well conserved among species. The largest chromosomes were found in *P. coccinea* and the smallest in *P. mixta* L.

In *P. alata*, *P. nitida*, and *P. mucronata*, there were two pairs of satellites located preferentially in the smaller chromosomes and in the long arm. In *P. coccinea* and *P. kermesina* up to three satellited pairs were visualised and up to eight in prometaphase cells of *P. alata*. In the single *P. coccinea* plant analysed, one satellite pair was much larger than any other observed (Fig. 2a).

The interphase nuclei of all 2n = 18 species analysed were semi-reticulate (Fig. 2d), except *P. mixta*. In this species, the interphase nuclei were areticulate, presenting a weakly stained diffuse chromatin and six to eight chromocentres. Meiosis was observed in four species. *P. cincinnata* Mast., *P. racemosa* Brot. and *P. setacea* DC. displayed nine bivalents and normal anaphase segregation (Fig. 5d), whereas a tetravalent was found in a few cells analysed of *P. jilekii* Wawra.

The third group of species, represented only by *P. foetida* L., displayed 2n = 20 small sized chromosomes in several populations analysed. The interphase nuclei were areticulate, with 16 to 18 chromocentres per nucleus. In meiosis, 10 bivalents and normal anaphases I and II were observed (Fig. 3f). One of the samples collected in Recife (PAS-193), in which only prophases and prometaphases were observed, appeared to have 2n = 22, due to the presence of distended proximal secondary constrictions (Fig. 4g, h). In a few cells of this sample, however, the number 2n = 20 was confirmed.

The analysis of eight *Passiflora* species with the fluorochromes CMA and DAPI revealed only one to three pairs of CMA^+ block and no $DAPI^+$ heterochromatin. In species with 2n = 12, a single $CMA^+/DAPI^-$ block was observed in the largest chromosome pair of *P. capsularis* L. and *P. rubra* L. and in the proximal region of two small to medium sized chromosome pairs of *P. tricuspis*. However, CMA^+ blocks stained weakly in *P. rubra*. In the group of 2n = 18, all CMA^+ blocks observed were terminally located. Three pairs were present in the chromosomes of *P. caerulea* (Fig. 5b), two pairs in *P. edulis* (Fig. 5e) and two pairs in *P. racemosa* plus one weakly stained and only sometimes observed. A single individual analysed of *P. amethystina* Mikan had four chromosomes with a small CMA^+ region and a single chromosome with a large one (Fig. 5c). Figure 5a and 5d show an anaphase I of *P. racemosa* uniformly stained with DAPI and an octoploid tapetal cell exhibiting several CMA^+ regions. In *P. foetida*, CMA^+ regions were observed in the telomere region of one chromosome pair and in the proximal region of two other pairs (Fig. 4g, h).

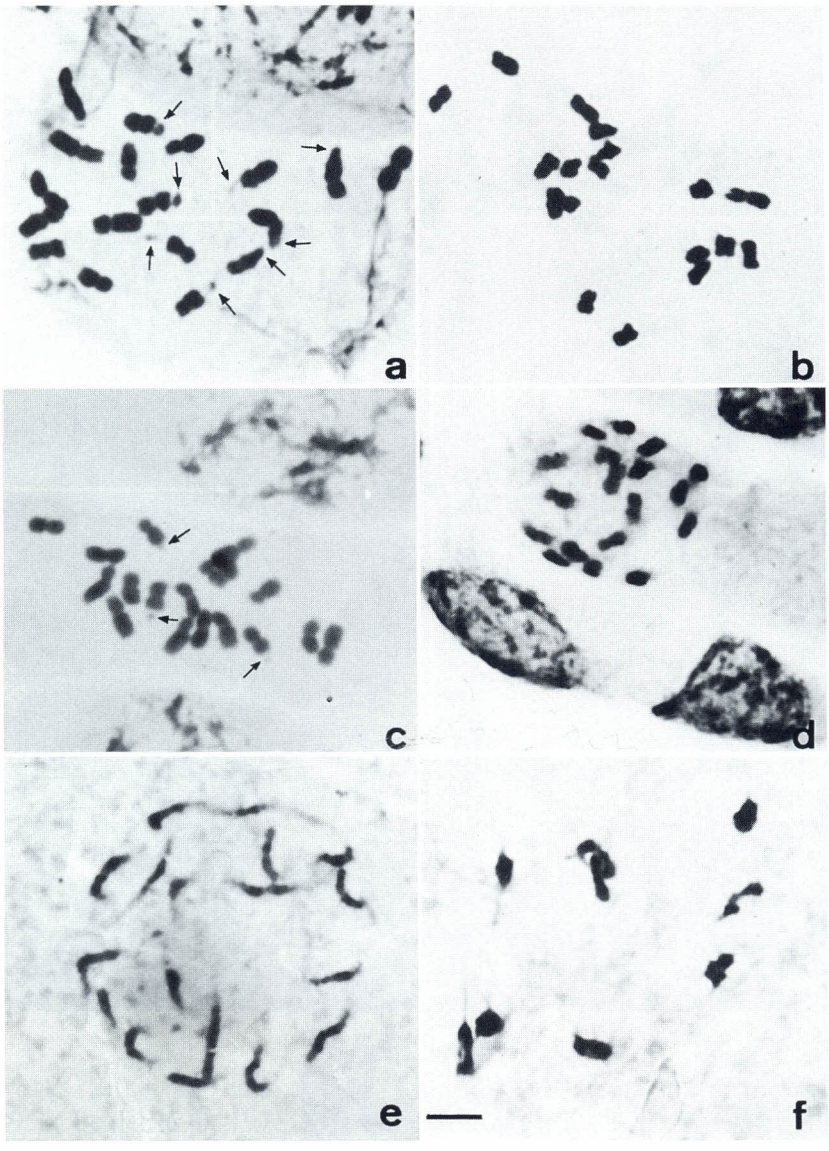

Fig. 2. Chromosome complements of *Passiflora* species with x = 9. **a** *P. coccinea*; **b** *P. kermesina*; **c** *P. nitida*; **d** *P. galbana*; **e** *P. setacea*; **f** *P. jilekii*. All complements are mitotic, except the meiotic metaphase I bivalents of *P. jilekii*. Note the semi-reticulate nuclei in **d**. Arrows in **a** and **c** point out satellites. Bar represents 5 μm

Discussion

Numerical chromosome variation

The analysis of the few cytogenetically known species of *Passiflora* divides the genus into three large groups: one with n = 6, 12, 18 constituted of the subgenera *Astephia* Killip, *Astrophea*, *Plectostemma*, *Pseudomurucuja* (Harms) Killip and *Psilanthus* (DC.) Killip; another with n = 10, restricted to the subgenus *Dysosmia*; and a third group with n = 9, 36, which includes cytologically well-known representatives of the subgenera *Calopathanthus*, *Distephana*, *Granadillastrum* (Triana & Planc.), *Passiflora*, *Tacsonia* and *Tacsonioides* (DC.) Killip. Table 2 presents the chromosome numbers reported for each subgenus. Within each groups there is a considerable stability in the karyological parameters, with only minor variations in the structure of the interphase nuclei, number of heterochromatic blocks and chromosome size. The only exceptions are 2n = 14, reported for *P. holosericea* L. and *P. lobata* (Killip) Hutch. ex J. M. MacDougal (Snow and MacDougal 1993), both belonging to the subgenus *Plectostemma* and possibly

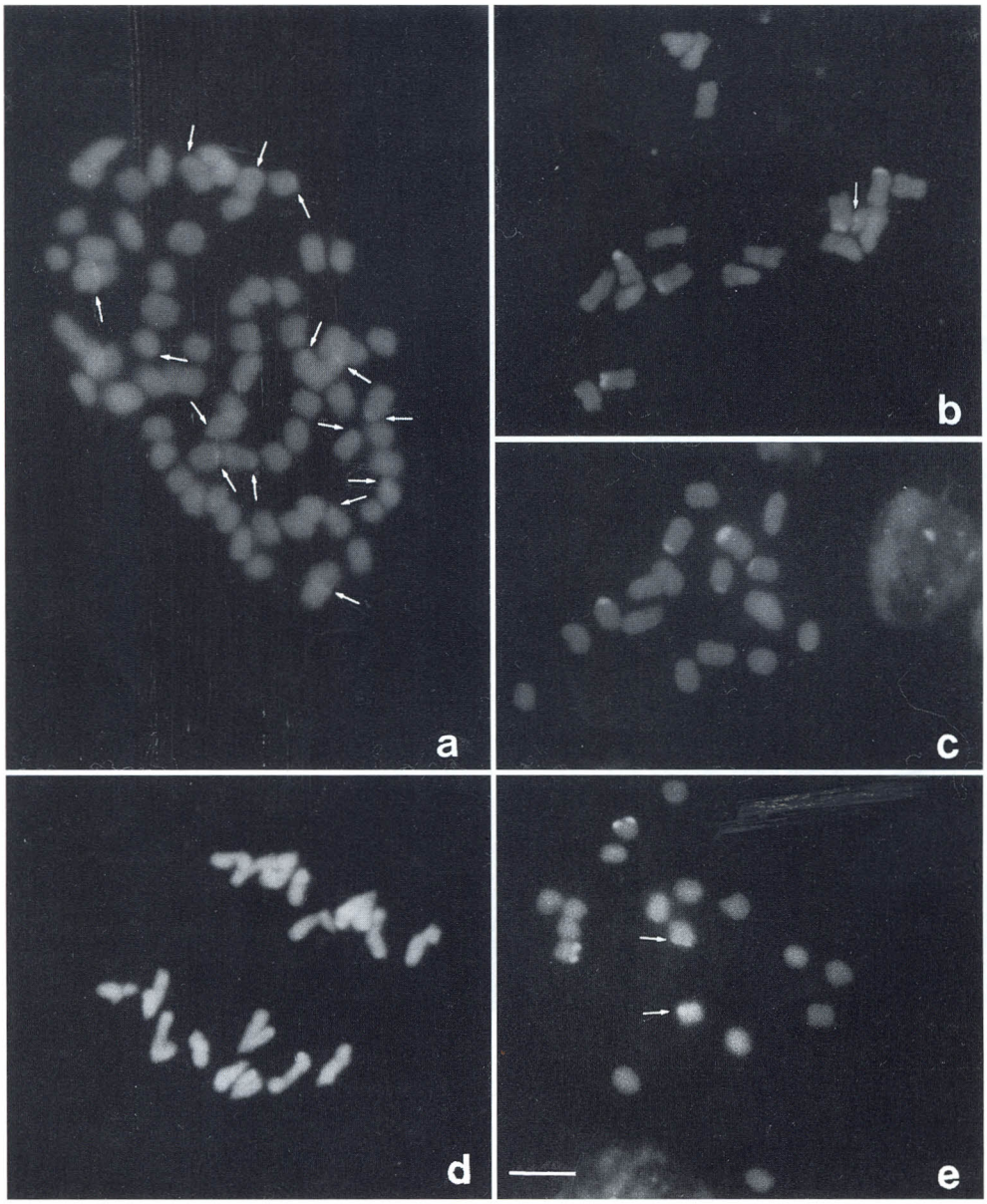

Fig. 5. CMA/DAPI double stained chromosome complements of *Passiflora* species with 2n = 18. **a** Octoploid tapetal metaphase of *P. racemosa* (2n = 8x = 72); **b** *P. caerulea*; **c** *P. amethystina*; **d** Anaphase I chromosomes of *P. racemosa*; **e** *P. edulis*. **a, b, c, e** CMA fluorescence; **d** DAPI fluorescence. Observe uniform staining with DAPI (**d**). Arrows point out weakly stained CMA blocks in **a, b** and **e**. Bar in **e** represents 5 μm

than in any other. It was observed in *P. suberosa* L., 2n = 12, 24, 36, *P. exsudans* Zucc., 2n = 24, *P. lutea*, 2n = 24, *P. tenuiloba* Engelm., 2n = 24 and *P. misera*, 2n = 12, 36. In the latter, the diploid sample had larger chromosomes than the hexaploid one, despite their general karyotype similarity, and the cytotypes were geographically very isolated, suggesting a possible speciation. Endopolyploid metaphases were commonly found in the anther tapetum of *Passiflora* species in general, and mixoploidy was found in root tips of *P. morifolia*, constituting potential sources of chromosome miscounts.

Table 2. Chromosome numbers known in *Passiflora* subgenera (according to Killip 1938)

Subgenera	Species[a]	Diploid numbers	References
Astrophea	*P. lindeniana* Triana & Planch.*; *P. pentagona* Mast.*	24	Berry 1987 Present work
Astephia, *Plectostemma*, *Pseudomurucuja*, *Psilanthus*	*P. aurantia* G. Forst.**; *P. aurantia* G. Forst. var. *aurantia*; *P. biflora* Lam.*; *P. bryonioides* Kunth; *P.* aff. *Candollei* Triana & Planch.; *P. capsularis* L.; *P. capsularis* L. var. *acutiflora* Hort.; *P. cinnabarina* Lindl.; *P. citrina* J. M. MacDougal; *P. cobanensis* Killip; *P. conzattiana* Killip; *P. coriacea* Juss.**; *P. costaricensis* Killip; *P. cubensis* Urban; *P. escobariana* J. M. MacDougal; *P. exsudans* Zucc.; *P. gilbertiana* J. M. MacDougal; *P. gracilis* J. Jacq. ex Link*; *P. herbertiana* Ker Gawl.**; *P. holosericea* L.; *P. juliana* J. M. MacDougal; *P. karwinskii* Mast.*; *P. lobata* (Killip) Hutch. ex J. M. MacDougal; *P. lutea* L.; *P. misera* Kunth; *P. morifolia* Mast.*; *P. nubicola* J. M. MacDougal*; *P. oaxacensis* J. M. MacDougal; *P. obtusifolia* Sessé & Moc. (syn. = *P. coriacea*); *P. penduliflora* Bertero ex DC.*; *P. perfoliata* L.; *P. porphyretica* Mast. var. *porphyretica*; *P. pulchella* Kunth** (syn. = *P. bicornis* Miller); *P. quinquangularis* S. Calderón (syn. = *P. capsularis*); *P. rovirosae* Killip; *P. rubra* L.; *P. sanguinolenta* Mast. & Linden; *P. standleyi* Killip; *P. suberosa* L.**; *P. tenuiloba* Engelm.; *P. tricuspis* Mast.; *P. warmingii* Mast.** (syn. = *P. morifolia*); *P. xiikzodz* J. MacDougal ssp. *itzensis*; *P. xiikzodz* J. M. MacDougal ssp. *xiikzodz*; *Passiflora* sp.*	12, 14, 24, 36	Beal 1969a; Oliveira 1996; Snow and MacDougal 1993; Turner and Zhao, 1992 *apud* Goldblatt and Johnson 1996; Present work
Calopathanthus, *Distephana*, *Granadillastrum*, *Passiflora*, *Tacsonia*, *Tacsonioides*	*P. actinia* Hook.; *P. alata* Dryand**; *P. amethystina* Mikan; *P. antioquiensis* H. Karst.**; *P. caerulea* L.**; *P. calcarata* Mast. (syn. = *P. subpeltata* Ortega)*; *P. cincinnata* Mast.**; *P. coccinea* Aubl.**; *P. cumbalensis* (H. Karst.) Harms var. *goudotiana* (Triana & Planch.) L. Escobar*; *P. edmundoi* Sacco; *P. edulis* Sims f. *flavicarpa*; *P. edulis* Sims**; *P. filamentosa* Cav.**; *P. foetida* L.**; *P. foetida* L. var. *fluminensis*** ; *P. foetida* L. var. *gossypifolia* (Desv.) Mast.**; *P. foetida* L. var. *hispida* Killip ex Gleason*; *P. galbana* Mast.; *P. giberti* N. E. Brown**; *P. glandulosa* Cav.; *P. incarnata* L.**; *P. jilekii* Wawra*; *P. kermesina* Link & Otto; *P. laurifolia* L.**; *P. ligularis* Juss.**; *P. magnifica* L. K. Escobar; *P. maliformis* L.**; *P. manicata* (Juss.) Pers.; *P. mixta* L.; *P. mollissima* (Kunth) Bailey**; *P. mucronata* Lam.; *P. nitida* Kunth**;	18, 20, 22, 36, 72	Beal 1969a; Dornelas and Vieira 1991; Mayeda and Vieira 1994; Mayeda and Vieira 1995; Snow and MacDougal 1993; Present work

Table 2 (continued)

Subgenera	Species[a]	Diploid numbers	References
	P. quadrangularis L.**; *P. racemosa* Brot.**; *Passiflora* sp. aff. *P. racemosa* Brot*; *P. seemannii* Griseb.**; *P. setacea* DC.*; *P. subpeltata* Ortega**; *P. tripartita* (Juss.) Poir.; *P. trisulca* Mast.; *P. umbilicata* (Griseb.) Harms; *P. vitifolia* Kunth; *Passiflora* sp. aff. *P. vitifolia* Kunth*; *P.* x *allardii*; *Passiflora* sp.		
Unknown	*Passiflora* sp. nov. A (L. Gilbert & J. MacDougal ined.)	18	Snow and MacDougal 1993

[a] Chromosome numbers were determined only in meiosis (*), only in mitosis (without asterisk), or in both (**)

The 38 species cytologically known in the subgenera with n = 6, 12 have short chromosomes, with only small karyotype differences. In the present work, the secondary constrictions were located in the terminal region of the largest chromosome pair in *P. capsularis* and *P. rubra* of section *Xerogona*, in the proximal region of one of the smallest pairs in *P. misera* and *P. tricuspis* of section *Decaloba* and in the terminal region of the smallest chromosome pair in *P. morifolia* of section *Cieca*. Beal (1973b) also reported two very similar satellited chromosome pairs in *P. aurantia* G. Forst. and *P. herbertiana*, both from section *Decaloba*. The analysis with the fluorochromes CMA and DAPI revealed a single $CMA^+/DAPI^-$ block apparently coinciding with the position of the secondary constrictions observed in these species, except by an extra CMA^+ block in *P. tricuspis*. Therefore, the number and position of secondary constrictions may be one of the most important karyological features in this group, as observed in some genera with stable chromosome numbers, like *Citrus* (Guerra et al. 1997) and *Hordeum* (Linde-Laursen et al. 1995). Since satellites are not always clearly distinguishable, analysis of a larger number of species with chromomycin A_3 or in situ hybridization with rDNA should provide better evidence of karyotype diversification within the group (see for example Cerbah et al. 1998).

Differences in karyotype symmetry between *Plectostemma* species are less conspicuous (Beal 1973a, Snow and MacDougal 1993). In the present work, the karyotype of most species was asymmetrical, but in some species, like *P. herbertiana* and *P. morifolia*, it was quite symmetrical, with meta- and submetacentric chromosomes of similar sizes.

The subgenus *Astrophea* has only two species analysed, both with n = 12. Since *Astrophea* comprises a further 60 species (Vanderplank 1996) it would not be surprising if diploid species with n = 6 were later found. In the system by Killip (1938), *P. lindeniana* Triana and Planch. was a representative of the arboreal species of the *Euastrophea* section,

while *P. pentagona* Mast. was a subscandent shrub, with old tendrils reduced to coarse spines, belonging to section *Pseudoastrophea*.

Karyology of the species with n = 9 and n = 10

The species with base number x = 9 presented larger uniformity in morphology and chromosome number. Polyploidy had only been previously registered in a cultivated form of *P. incarnata* L. (2n = 36, Lloyd 1963), although the diploid form is more common (Table 2). In the present work, the occurrence of octoploidy (2n = 72) in an unidentified species is reported. All other countings for this group displayed 2n = 18.

In spite of the stability in chromosome number, there are significant karyotype variations among species of this group. Beal (1973a) observed that the chromosomes of *P. seemanni* Griseb. were ca. 20% larger than those of *P. maliformis* L. Oliveira (1996) found the haploid complement of *P. cincinnata* 30% longer than that of *P. coccinea*. Snow and MacDougal (1993) drew attention to a 2n = 18 species (*Passiflora* sp. nov. A) with chromosome size similar to the species with n = 6, which usually have chromosomes shorter than those of n = 9. In the present work, the largest chromosomes were found in *P. nitida* and the smallest in *P. mixta*, although these differences were largely influenced by pre-treatment and condensation degree at metaphase.

The karyotypes with 2n = 18 also differ in number and position of secondary constrictions. In seven species of the present sample, secondary constrictions were observed mainly at a terminal or sub-terminal position in the long arm of two or three chromosome pairs. Snow and MacDougal (1993), Dornelas and Vieira (1991) and Oliveira (1996) found one or two satellite pairs in several species of this group, whereas Beal (1973b) registered up to seven satellite pairs in *P. quadrangularis* L. The analysis with CMA and DAPI in *P. racemosa*, *P. edulis* and *P. caerulea* showed CMA^+ blocks in the terminal region of two or three chromosome pairs, apparently coinciding with the position of the satellites.

The only species with n = 10, *P. foetida*, is a weed with wide geographical distribution throughout the Americas, great morphological diversity and dozens of varieties (Killip 1938). However, it is karyologically very stable, without any demonstrated polyploidy, dysploidy or meiotic irregularity. Its small sized chromosomes and areticulate interphase nuclei, are similar to the species with n = 6, although its higher karyotype symmetry, the high number of CMA^+ blocks and the chromosome number itself suggest a greater proximity to the species with n = 9. Lorenz et al., from the Genetics Department of Universidade Federal do Rio Grande do Sul, Brazil (personal communication), based on cpDNA data, found it closer to the x = 9 group than to those with x = 6. Karyologically, however, *P. foetida* appears quite isolated. It would be very important to know the chromosome number of other species that compose the subgenus *Dysosmia*.

The cytotaxonomy of the group

The most difficult task in the cytotaxonomical analysis of a group is the identification of its base number. Although only about 17.8% of the *Passiflora* species have been investigated cytologically, its main chromosome numbers are assumed to be n = 6 and n = 9. The central question is how these numbers are related to each other and which one is the base number. The hypothesis of $x_1 = 3$ for the genus, admitted by Storey (1950), would help to understand the strictly numerical relationships between these numbers. However, there is no karyological indication suggesting that the species with n = 6 and n = 9 are polyploids derived from an ancestral group with three chromosomes. Furthermore, the absence of n = 3 in the genus, in the family or in any known representative of Violales makes this hypothesis unacceptable. Morawetz (1986) considered x = 6 as the base number of the family and the species with 2n = 18 as

diploidised triploids. However, the capacity to form normal bivalents and the occurrence of pairs of satellites and CMA^+ blocks in these species argue definitively against this hypothesis.

Based on few counts, Raven (1975) considered that the base number of *Passiflora* could be x = 9. Snow and MacDougal (1993) accepted n = 9 as the most primitive haploid number, based on the fact that the karyotypes with n = 9 are more symmetrical than those with n = 6, which according to Stebbins (1971) would be an indication of primitiveness. However, some species with n = 6 have symmetrical karyotypes, suggesting that it is not a reliable indicator of the ancestrality of n = 9.

The base number has been assumed by different authors from a number of distinct criteria. Guerra (2000), reviewing this subject, defined base number as one of the haploid numbers present in a taxon that most parsimoniously explains the chromosomal variability of that group and has a clear relationship with the base numbers of its most closely related taxa. In this case, x = 6 or x = 12 could be the base number for the genus and the family. The number x = 12 is well documented in two of the three cytologically known subgenera of *Passiflora* as well as in some other genera of Passifloraceae. *Adenia* Forssk., the second largest genus, has n = 12 and n = 24, *Tetrapathaea* (DC.) Rchb. has n = 12, and *Deidamia* Noronha ex Thouars and *Crossostemma* Planch. ex Hook. have n = 11. However, the change from n = 12 to n = 6 in the genus *Passiflora* would require a series of six steps by descending dysploidy, and at least two of them (n = 8, n = 11) were not found. Furthermore, the interruption of the series precisely at n = 6, a successful group of species

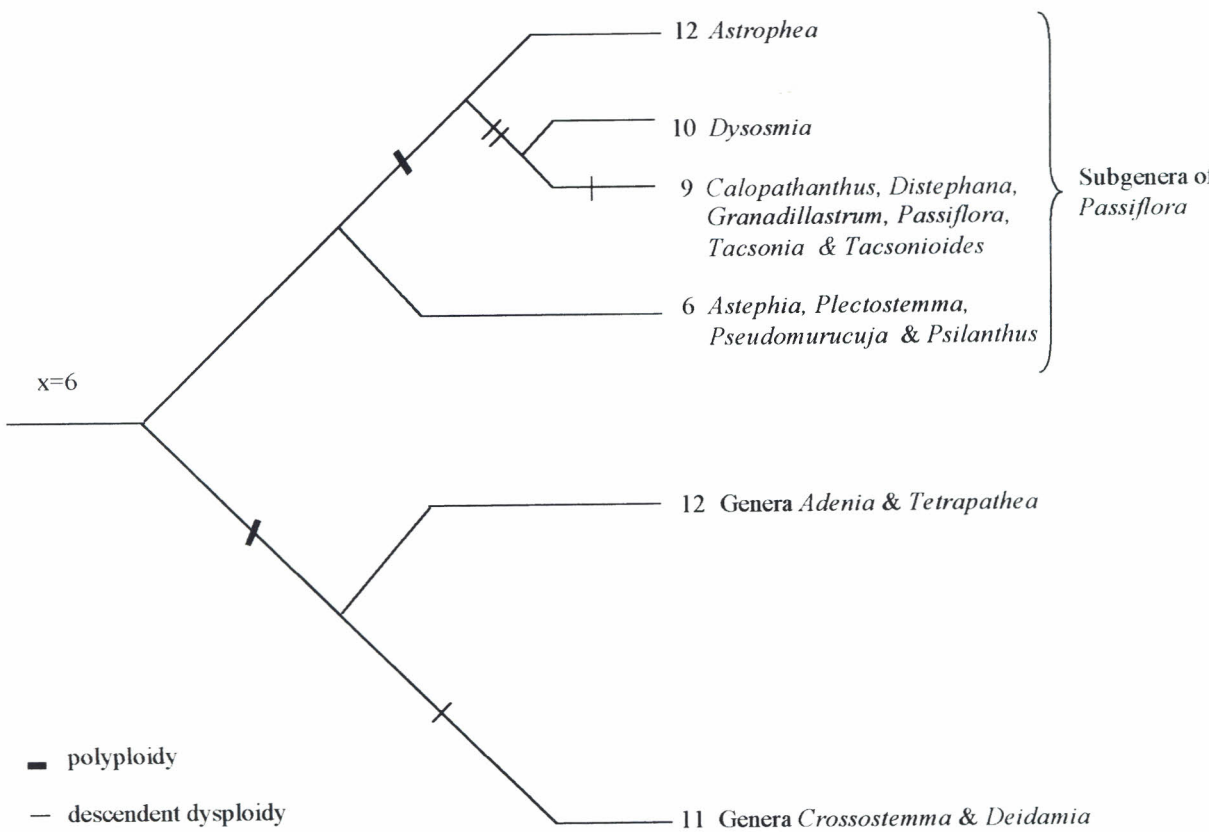

Fig. 6. Probable relationships among main haploid numbers known in *Passiflora* subgenera and other genera of Passifloraceae

including some polyploids with n = 12, points to n = 6 as the original number. In this case, n = 12 in subgenus *Astrophea* and in the genera *Adenia* and *Tetrapathaea* would be of polyploid origin, based on x = 6. Species with n = 6 are also found in Violaceae (Fabijan et al., 1987), whereas n = 5 and n = 7 are found in Turneraceae (Solis-Neffa and Fernandez 1993, Lavia and Fernandez 1993), two families closely related to Passifloraceae. Figure 6 illustrates a cladogram based on x = 6, which is more parsimonious than that based on x = 12. All Passifloraceae haploid numbers may be linked to x = 6 through dysploidy and polyploidy, the simplest and most widespread evolutionary events known in angiosperms (Stebbins 1971, Grant 1982).

Snow and MacDougal (1993) considered n = 12 in *P. lindeniana* of *Astrophea*, a subgenus with some primitive characteristics, as a hindrance to the hypothesis of x = 9. However, the most primitive representatives of a taxon frequently do not possess n = x, but rather a dysploid or a polyploid variant of the base number (Smith-White 1959, Guerra 2000). Therefore, the occurrence of $x_2 = 12$ in one of the most primitive subgenera of *Passiflora* does not conflict with the hypothesis of $x_1 = 6$ in the genus. On the contrary, to explain the diversity in chromosome number of the family based on x = 9, a much larger number of steps would be necessary. The species with n = 9 are karyotypically very similar, suggesting they have had a single origin, probably by descending dysploidy from n = 12 or from a species similar to *P. foetida*, with n = 10. Alternatively, they could have arisen by ascending dysploidy from n = 6, although this is a less common evolutionary mechanism (Grant 1982).

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