

Chromosome Counts on Angiosperms Cultivated at the National Tropical Botanical Garden, Kaua'i, Hawai'i¹

MICHAEL KIEHN² AND DAVID H. LORENCE³

ABSTRACT: Karyological data for 30 collections of angiosperms cultivated at the National Tropical Botanical Garden representing 29 species (mainly Hawaiian or Pacific endemics) from seven families (mostly Rubiaceae) are presented, including first reports for the genera *Anthocephalus*, *Bobea*, *Calycostia*, and *Cyclophyllum* (Rubiaceae). Results are compared with previously published data, and their systematic significance is discussed.

CHROMOSOME NUMBERS and/or ploidy levels often provide important information about the phylogeny, evolution, and relationships among plant groups. However, karyological knowledge of tropical plants is still very limited. The National Tropical Botanical Garden (NTBG) on Kaua'i holds a large living collection of taxa with tropical and subtropical distribution, including numerous rare and endangered species of the Hawaiian Islands. Many of these taxa have never been investigated karyologically. The first results of chromosome studies on such taxa are presented herein.

MATERIALS AND METHODS

During four visits by M.K. to the NTBG, fixation of meristematic tissues (actively growing root tips or apices for counts of mitotic chromosome numbers, young flower buds for meiotic investigations) of plants cultivated by the NTBG were made in a freshly mixed 3:1 solution of ethanol (96%):glacial acetic acid. The fixed material was kept under cooled conditions before staining with Giemsa (after hydrolysis in 5 N HCl for 50 min at 20°C) or with Feulgen

(see Kiehn [1995] for details). In cases of insufficient staining by these techniques, hot aceto-carmin (2% solution in 45% acetic acid) was used additionally. Exact counts could not be achieved in several cases because of limited material, high chromosome numbers, and/or chromosomes clumping together (possibly as a result of the presence of tannins). Thus, a range of chromosome numbers is given in those cases. Collection data and techniques used for each species are listed in Table 1. Voucher specimens were made for most taxa and have been deposited in the herbaria at Vienna University (WU), at the National Tropical Botanical Garden (PTBG), and/or in other herbaria as stated in Table 1. Permanent slides for most counts are in the collection of M.K.

RESULTS

Araliaceae: The result of $2n = 48$ for *Munroiodendron racemosum* (C. Forbes) Sherff (monotypic genus endemic to Hawai'i) is in accordance with the report of $n = 24$ for the same species by Carr (1978). Thus, *Munroiodendron* has the same basic number ($x = 12$) and ploidy level ($4x$) as the only counted species of the closely related genus *Tetraplasandra*: *T. oahuensis* (A. Gray) Harms [Carr 1978, as *T. cf. meiantra* (Hbd.) Harms].

Asteraceae: The only available chromosome number report for the genus *Faujasia* is $2n = 20$ for the Mauritian *F. flexuosa* (Lam.) Jeffrey [Beuzenberg 1975, as *Faujasia*

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² Institute of Botany, University of Vienna, Rennweg 14, A-1030 Vienna, Austria.

³ National Tropical Botanical Garden, P.O. Box 340, Lāwā'i, Kaua'i, Hawai'i 96765.

TABLE 1
CHROMOSOME NUMBERS OF ANGIOSPERMS CULTIVATED AT THE NTBG

TAXON	TECHNIQUE	<i>n</i>	<i>2n</i>	NTBG NO.	ORIGIN; VOUCHER (LOC. OF VOUCHER)
Araliaceae					
<i>Munroiodendron racemosum</i> (C. Forbes) Sherff	F + AC	—	48	910244	U.S.A.: Hawai'i, Kaua'i, NTBG, Lāwa'i Valley, cultivated; DL 7628 (PTBG)
Asteraceae					
<i>Faujasiopsis reticulata</i> (Vahl) C. Jeffrey	F + AC	—	20–24 ^a	910340	Mascarenes: Mauritius, Mondrain; DL 7080
Gesneriaceae					
<i>Chirita moonii</i> Gardn.	G	—	18 ^a	890495	Sri Lanka: coll. S. Lucas s.n.; MK 900829-1/3 (PTBG; wu)
Rhamnaceae					
<i>Gouania hillebrandii</i> Oliv.	F + AC	(23)–24 ^a	—	920145	U.S.A.: Hawai'i, Maui, Līhau; DL 7609 (PTBG, wu)
Rubiaceae					
<i>Anthocephalus chinensis</i> (Lamk.) A. Rich. ex Walp.	G	—	44 ^b	831086	China: Mengla, seeds from Yunnan Inst. Trop. Botany; DL 7608 (PTBG), MK 900904-1/1 (wu)
<i>Bohea elatior</i> Gaud.	F	—	66–70 ^b	890518	U.S.A.: Hawai'i, Kaua'i, Limahuli, C. Wichmann 197 (PTBG)
<i>timonioides</i> (J. D. Hook.) Hillebr.	G	—	88 ^b	910017	U.S.A.: Hawai'i, Kaua'i, K. Wood 291 (AD, MU, PTBG)
<i>Calycosia sessilis</i> A. Gray	F	—	44 ^b	890777	W. Samoa: Upolu; T. Flynn 3718 (PTBG)
<i>Canthium coromandelicum</i> (Burm. f.) Alston	G	—	44	830087	Sri Lanka: coll. S. Lucas 1004; MK 900829-1/5 (wu)
<i>Chiococca coriacea</i> M. Martens & Galeotti	F, G	—	26 ^a	880087001	Mexico: Veracruz; DL 6639 (BISH, PTBG, US), MK 900914-4/1 (PTBG, wu)
<i>Cyclophyllum barbarum</i> (Forst. f.) F. Hallé & J. Florence var. <i>marquesensis</i> (F. Br.) Fosb.	G	—	44 ^a	880609	Marquesas Is.: Fatu Hiva; MK 900820-1/16 (wu), DL 6133 (BISH, PTBG, US)
<i>Gardenia brighamii</i> H. Mann	F	—	22 ^a	910022	U.S.A.: Hawai'i, Lāna'i, Lapaiki; S. Perlman 11041, DL 7629 (PTBG)
<i>remyi</i> H. Mann	F	—	22 ^a	870955	U.S.A.: Hawai'i, Kaua'i, Limahuli; T. Flynn 2664 (PTBG)
<i>Guettarda uruguensis</i> Cham. & Schlecht.	F	—	≤ 88 ^a	890647	Brazil: Rio Grande do Sul, seeds from HB Porto Alegre; MK 900829-1/15 (wu)

<i>Haldina</i> <i>cordifolia</i> (Roxb.) Ridsd.	G	—	44	750166001	Australia: Queensland; DL 6619 (PTBG, wu), MK 900912-1/1 (PTBG, wu)
<i>Ixora</i> <i>amplifolia</i> A. Gray	G	—	22 ^a	890781	W. Samoa; T. Flynn 3711 (MO, PTBG, US)
<i>samoensis</i> A. Gray	G	—	22 ^a	800495001	W. Samoa: coll. A. Whistler s.n.; MK 900829-1/9 (PTBG, wu)
<i>Morinda</i> <i>trimeria</i> Hillebr.	G	—	22 ^a	905057	U.S.A.: Hawai'i, O'ahu, Kaluaa Gulch; MK 900818-1/1, S. Perlman 5822 (PTBG)
<i>Mussaenda</i> <i>raiateensis</i> J. W. Moore	G	—	22 ^a	870052	Tonga: Vavau; DL 6428 (PTBG), MK 900829-1/1 (PTBG, wu)
<i>Nauclea</i> <i>orientalis</i> (L.) L.	G	—	88	830434	Sri Lanka: coll. S. Lucas 1201; DL 7588 (PTBG, wu)
<i>Psychotria</i> <i>brachyceras</i> Muell.-Arg.	G	—	22 ^a	890644	Brazil: Rio Grande do Sul, seeds from HB Porto Alegre; MK 900829-1/12 (seeds: wu)
<i>erythrocarpa</i> Schlecht.	G	11 ^a	—	820243001	Mexico: Chiapas; MK 900829-1/4 (wu)
<i>hobbyi</i> Sohmer	G	—	88 ^a	905074	U.S.A.: Hawai'i, Kaua'i, Mahanaloa; S. Perlman 10819 (=S. Perlman 10369; MO, PTBG)
<i>insularum</i> A. Gray	F	—	ca. 110 ^a	890774	W. Samoa: Upolu; T. Flynn 3723 (PTBG)
	F	—	ca. 110 ^a	890776	W. Samoa: Upolu; T. Flynn 3722 (PTBG)
<i>kaduana</i> (Cham. & Schlecht.) Fosb.	G	—	66 ± 2 ^a	890520	U.S.A.: Hawai'i, Kaua'i, Limahuli; T. Flynn 3531 (PTBG)
<i>Randia</i> <i>truncata</i> Greenm. & C.H. Thomps.	G	—	22 ^a	860199	Mexico: seeds from HB UNAM (Mexico City); MK 900829-1/7 (PTBG, wu)
<i>Rosenbergiodendron</i> <i>formosum</i> (Jacq.) F. Fagerl.	G	—	22 ^a	710061	Horticult. origin; MK 900829-1/11 (PTBG, wu)
Rutaceae					
<i>Zanthoxylum</i> <i>hawaiiense</i> Hillebr.	G	—	136–144	905317	U.S.A.: Hawai'i, Hawai'i, Pōhakuloa; S. Perlman 11181 (wu)
Saxifragaceae (Brexiaaceae)					
<i>Brexia</i> <i>madagascariensis</i> Thouars	G	—	60 ± 1 ^c	710052	Horticult. origin; DL 5719 (PTBG), MK 900829-1/8 (wu)

Voucher numbers: MK, Michael Kiehn; DL, David Lorence. Additional abbreviations of herbaria: AD = State Herbarium, Adelaide, Australia; BISH = Herbarium, Bishop Museum, Honolulu, Hawai'i, U.S.A.; MO = Herbarium, Missouri Botanical Garden, St. Louis, Missouri, U.S.A.; MU = Herbarium, Botany Department, Miami University, Oxford, Ohio, U.S.A.; US = Herbarium, Smithsonian Institution, Washington, D.C., U.S.A.

^a First report for species.

^b First report for genus.

^c Deviates from literature data.

flexuosa (Lam.) Baker]. A photo in Beuzenberg (1975) shows four large satellites in the diploid chromosome set. These satellites might be the reason for the range of $2n = 20$ – 24 reported here for *Faujasiopsis reticulata* (Vahl) C. Jeffrey from Mauritius.

Gesneriaceae: *Chirita moonii* Gardn. from Sri Lanka shows $2n = 18$ as do most investigated *Chirita* species (see survey by Skog 1984).

Rhamnaceae: In the investigated *Gouania hillebrandii* Oliv. (a Hawaiian endemic, federally listed as endangered), a tendency of the mitotic and meiotic chromosomes to clump together made mitotic counts impossible. Although a meiotic number of $n = 23$ could not be excluded with certainty, $n = 24$ appears more probable from our investigations, particularly because $n = 24$ (based on $x = 12$) seems not to be unusual in the Rhamnaceae (Carr 1978). However, Carr (1978) reported $n = 23$ for *Gouania gagnei* St. John (now a synonym of *G. meyenii* Steud. [cf. Wagner et al. 1990]).

Rubiaceae: All Naucleaeae-Adininae investigated cytologically exhibit $x = 11$ and tetraploidy, as do our counts for *Anthocephalus chinensis* (Lamk.) A. Rich. ex Walp. (first chromosome number record for the genus in the revised form of Ridsdale 1978) and *Haldina cordifolia* (Roxb.) Ridsd. (result in accordance with the literature for this species [see survey in Kiehn 1986]). In the Naucleaeae-Naucleinae also most counts reveal tetraploidy based on $x = 11$. The only known exception is *Nauclea orientalis* (L.) L., for which octoploidy is here confirmed.

In the Guettardeae, only tetraploid taxa (based on $x = 11$) have been detected so far. Thus, the records of hexa- and octoploidy for two *Bohea* species (an endemic Hawaiian genus) and the octoploidy of *Guettarda uruguayensis* Cham. & Schlecht. reported here are the first exceptions.

For the Samoan endemic *Calycosia sessilis* A. Gray, both basic number ($x = 11$) and ploidy level ($4x$; polyploidy is often found in Psychotrieae [Kiehn 1986]) are in accordance with those of other Psychotrieae already investigated karyologically. Furthermore, other karyological characters (e.g., chromosome

structure and Giemsa staining pattern [resulting in dark blue, somewhat "color-coated" chromosomes, which is typical for *Psychotria* chromosomes (M.K., unpublished data)]) can be interpreted as indications for a close relationship of this taxon to the genus *Psychotria*. *Psychotria* species counted in the course of this study come from different areas: *P. brachyceras* Muell.-Arg. and *P. erythrocarpa* Schlecht. are New World taxa (South American and Mexican, respectively). A revision for *Psychotria* for that area is still lacking, so their diploidy on $x = 11$ cannot be put into a systematic or evolutionary context. More interesting are the results for the Pacific *Psychotria* species. The first counts reported for Hawaiian *Psychotria* reveal the existence of (at least) two different ploidy levels ($6x$ and $8x$). Because *P. mariniana* (Cham. & Schlecht.) Fosb., which is morphologically closely related to and possibly hybridizing with *P. kaduana* (Cham. & Schlecht.) Fosb. (Wagner et al. 1990; M.K., pers. obs.), also exhibits hexaploidy with $2n = 66$ [Coll. data: U.S.A.: Hawai'i, O'ahu, Ko'olau range, Mānana Trail; 17.7.1989; M. Kiehn et al. MK-890717-1/1 (BISH, PTBG, WU)] (M.K., unpublished data), the different ploidy levels might support the idea of different colonizations of *Psychotria* in Hawai'i. The two Samoan accessions of *P. insularum* A. Gray both are high polyploids (possibly $10x$), indicating a derived status of this taxon regarding chromosomes. There are only two other counts available for *Psychotria* from Samoa (Whistler 1986): *P. geminodens* K. Schum. is diploid with $n = 11$, and *P. closterocarpa* A. Gray is tetraploid ($n = 22$). Because only the latter is probably related to *P. insularum* (Whistler 1986), the divergent ploidy levels still cannot be discussed in terms of colonization or speciation patterns.

The reports of $2n = 44$ for *Cyclophyllum barbarum* (Forst. f.) F. Hallé & J. Florence var. *marquesenensis* (F. Br.) Fosb. (first count for the genus) and *Canthium coromandelicum* (Burm. f.) Alston match the data already known for the tribe Vanguerieae (Kiehn 1995). With $2n = 26$, *Chiococca coriacea* M. Martens & Galeotti exhibits a basic number ($x = 13$) unusual in Rubiaceae. The other re-

ported basic numbers in the genus are $x = 11$ (doubtful), $x = 12$, and $x = 14$ (Kiehn 1995). This might indicate that actual changes in basic numbers are taking place in the genus. The same pattern is found in the genus *Exostema* (basic numbers $x = 12, 13, 14$ [Kiehn 1995]), which has recently been included in the tribe Chiococceae (Bremer 1992). $x = 12$, another unusual basic number in Rubiaceae, is also present in the genera *Portlandia* and *Cubanola*, which also have been included in Chiococceae by Bremer (1992). The possibility of a closer relationship between those taxa should be studied further. *Catesbaea*, which shows structurally similar chromosomes and also has $x = 12$ as its basic number, might also be included in such investigations. All Gardenieae species (two Hawaiian endemic *Gardenia* species, the Mexican *Randia truncata* Greenm. & C. H. Thoms., and *Rosenbergiodendron*), the two counted Samoan endemic *Ixora* species, and the Pacific *Mussaenda raiateensis* J. W. Moore are all diploids with $2n = 22$, as are the majority of their karyologically known relatives. *Morinda trimera* (a Hawaiian endemic) also has $2n = 22$ as do most other karyologically known *Morinda* species except *M. citrifolia* L., which is tetraploid.

Rutaceae: The cytology of Rutaceae has been studied in detail by Guerra (1984). He mentioned stickiness of chromosomes in higher polyploid *Zanthoxylum* species. This is also the reason for the range of $2n = 136$ – 144 obtained for *Z. hawaiiense* Hillebr. (Table 1), the first count for a Hawaiian member of the genus. This species belongs to the highest known ploidy level in the genus, with chromosome numbers for *Zanthoxylum* reported from $2n = 32$ to $2n = 136$ (Guerra 1984). A basic number of $x = 18$ has been established for the four Hawaiian species of Rutaceae previously counted. Two *Platydesma* species (*P. cornuta* Hillebr.: $n = 18$ [Carr 1978]; *P. rostrata* Hillebr.: $2n = 36$ [Guerra 1984], $n = 18$ [Carr 1985a]) are diploids, whereas one of the two investigated *Melicope* species exhibits diploidy (*M. elliptica* A. Gray: $n = 18$ [Carr 1978 as *Pelea elliptica* (A. Gray) Hillebr.] and the other tetraploidy (*M. wawraeana* (Rock) T. Hartley & B. Stone: $2n = 72$ [Guerra 1984

as *Pelea wawraeana* Rock]). Thus, *Z. hawaiiense* has by far the highest known chromosome number of any Hawaiian Rutaceae.

Saxifragaceae (Brexiaaceae): There is one count for *Brexia madagascariensis* Thouars in the literature: Schoennagel (1931) reported $2n = 64$ for a plant cultivated at the Botanical Garden of Kiel (Germany). He mentioned that the chromosomes were extremely small but elongated; he also used a microtome cutting technique for obtaining his counts. Probably due to these facts his result ($2n = 64$) is slightly higher than ours ($2n = 60 \pm 1$). However, this diploid number for *B. madagascariensis* is quite unusual in Saxifragaceae.

DISCUSSION

Stuessy and Crawford (1992:163) stated that there is "chromosomal stasis during speciation of angiosperms in oceanic islands." That is, after successful establishment of a new colonizer (which might include changes in chromosome basic number and/or ploidy levels compared with its ancestry), virtually no further chromosomal changes should take place in species deriving from that taxon. The hypothesis was based on a statistical analysis of changes in chromosome basic numbers and ploidy levels for angiosperms from different oceanic islands, especially from the Hawaiian Islands and the Juan Fernández Islands (Stuessy and Crawford 1992).

If this hypothesis has merit, no (or very few) cases of different basic numbers or ploidy levels should be found in groups that have speciated on Pacific Islands. The situations in *Psychotria* and especially in *Bohea*, however, are contradictory to this hypothesis. Ploidy levels most probably higher than in the ancestral groups are present in the taxa studied here, and different ploidy levels must have evolved in the Hawaiian Islands. A well-documented example of chromosomal evolution in the Hawaiian flora involving dysploidy and several homoploid structural changes is the *Argyroxiphium-Dubautia-Wilkesia* alliance (Asteraceae-Madiinae) (Carr 1985b, Carr and Kyhos 1981, 1986). The

observations reported here have several possible consequences with respect to the question of chromosomal stasis versus change in taxa on oceanic islands. The observed limitation of chromosomal variation in island-evolved taxa may be related to already limited chromosomal variation of the originating extra-island groups (e.g., *Cyrtandra* [Luegmayer and Kiehn 1991; M.K., unpublished data]). Potential variation both in ploidy level and/or basic numbers may exist in groups speciating on islands if the continental ancestors exhibit variation in this regard (e.g., *Psychotria* [Kiehn 1986]). It is remarkable that chromosome numbers of oceanic island representatives are the highest ones known in several cases (e.g., *Ilex*, *Pisonia*, *Coprosma ernodeoides* A. Gray, *Hedyotis* spp. in Hawai'i [Carr 1978, 1985a, Skottsberg 1955, Kiehn 1993; M.K., unpublished data]). The question remains unresolved: at what point in evolution has polyploidization taken place in these cases? Or, does polyploidy provide an advantage when a new area is colonized? Further studies are necessary to clarify this situation, at least in the Hawaiian Islands.

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