CHROMOSOME NUMBERS IN THE ANNONACEAE [1]

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CHROMOSOME NUMBER data on the Annonaceae are valuable for two reasons: first, tropical fruit growers have been paying considerable attention to several species of the family (for example, Fairchild, 1943, and Popenoe, 1945); and second, the family Annonaceae has an interesting phylogenetic position and geographical distribution, Rehder, 1940. The purpose of the present paper is to contribute new information and summarize the available data on the Annonaceae.

Hutchinson (1923) listed ninety-five genera in the Annonaceae but considered that a future monographer would probably reduce the number. Rehder (1940) estimated that there are more than 70 genera and more than 600 species in the family. They are native to the Tropics of the Old and New Worlds and some of them are widely cultivated in the Tropics as fruit and shade trees. Well-known tropical fruits are the Sour-Sop, *Annona muricata* L.; Custard-Apple, *Annona reticulata* L.; Sweet-Sop, *Annona squamosa* L.; and the Cherimoya, *Annona Cherimola* Mill. *Annona glabra* L. is used as a grafting stock; and *Polyalthia longifolia* (Lam.) Hook. f. is used as an avenue tree in India. Perfume is made from the flowers of *Artabotrys odoratissimus* R. Br. and *Cananga odorata* (Lamb.) Hook. f. & Thoms.

The genus *Asimina* is native to North America and can be divided into two groups (Small, 1926). In the first group, there are *Asimina triloba* (L.) Dunal which occurs from the Great Lakes Region to eastern Texas and northern Florida, and *Asimina parviflora* (Michx.) Dunal which occurs from northern Florida to North Carolina and Mississippi. *Asimina triloba* often forms thickets but isolated trees are over 30 ft. in height. *A. parviflora* is not as winter hardy as *A. triloba;* a population of seedlings of *A. parviflora* from North Carolina has consistently shown winter injury in the plots at The Blandy Experimental Farm, Boyce, Virginia (unpublished data, author and Dr. Orland E. White). In the second group are *Asimina pygmaea* (Bartr.) Dunal and seven allied species, all shrubs, which are native to Florida and vicinity.

CYTOLOGICAL DATA.—Cytological investigations of species of Annonaceae have been limited. Much effort is required to assemble the material and the determinations of chromosome number in many of the species require numerous observations. An understanding of the morphology of the chromosomes is a prerequisite to correct counting in species with a range of chromosome size. In this paper, the methods described by Bowden (1945 a,b; 1948) were used for the new determinations. Seeds of the North American species were stratified at a temperature a little above freezing while seeds of tropical species were planted in a warm greenhouse as soon as received. Root-tip material for these new counts was obtained in 1941–42 and in 1946. The photomicrographs were made by use of a Spencer research microscope equipped with a 90 X oil immersion objective, N.A. 1.30, and 15 X ocular, in conjunction with Wratten filters No. 60 (green) and E No. 22 (deep orange).

All of the slides of annonaceous material prepared by the author from 1940 to 1947 were recently reexamined. Some of the five to seven-year old slides were still fairly well stained (fig. 1–4, 7–8). The range in chromosome size made many examinations necessary in order to determine the morphology of the chromosomes so that accurate interpretations could be made. There was a ten-

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dency for some of the chromosomes to clump or stick to one another, and for a few chromosomes to lie obliquely or vertically. The only exception was *Annona glabra;* the chromosomes of this species did not show such a marked tendency to clump together and numerous metaphases were observed in which the 28 chromosomes were well separated.

Bowden (1945a) figured the chromosome complements of nine species of Annonaceae. Some of the species showed one pair of large chromosomes and one small pair, *e.g., Asimina triloba* and *Annona muricata.* Fig. 5 and 6 of the present paper show the chromosome complement of *Annona Cherimola* Mill. In fig. 5, the two small chromosomes are at 4 and 10 o'clock; in fig. 6, they are at 2 and 3 o'clock. One large pair of chromosomes and another pair almost as large can be recognized. The two small chromosomes frequently stuck to or were hidden by larger chromosomes, so that numerous metaphases were examined in order to determine the chromosome number.

In the following compilation, all available published data are included as well as new data which have resulted from the author's investigations in 1941–42 and 1946. The nomenclature and classification of the genus *Annona* follow that of Safford (1914); and the nomenclature of the genus *Asimina* is that of Fries (1939).

FAMILY ANNONACEAE RICH.

ASMINA ADANS.

Asimina triloba (L.) Dunal.—North American Papaw; Eastern North America; 1. trees from Mississippi State College, and Smith, Indiana, $n = 9$, Locke (1936); 2. collections at the Blandy Experimental Farm, Boyce, Virginia, grown from seed from New York, Kentucky, Maryland, Michigan, Ohio, and Indiana, $n = 9$, $2n = 18$, Bowden (1945a); 3. 200 seedlings in 23 populations from New York and Ontario, west to Nebraska and Kansas, south to Arkansas and east to North Carolina, $2n = 18$, Bowden (1948). 4. five triploid mutant seedlings, one each from Queenston, Ontario; Geneva, New York (cultigen); Brockport, New York; Front Royal, Virginia; and Clay City, Kentucky, $2n = 27$, Bowden (1948).

Asimina parviflora (Michx.) Dunal. — Small-fruited Papaw; Northern Florida to North Carolina and Mississippi; 1. col. by Dr. W. C. Coker at Chapel Hill, North Carolina, $2n = 18$, Bowden (1945a).

Asimina incana (Bartr.) Exell (= *A. obovata* (Willd.) Nash).—Flag-Papaw; Florida; 1. col. by Erdman West, Gainesville, Florida, $2n = 18$, Bowden (1945a); 2. two more populations from same source, $2n = 18$; 3. from Dr. G. A. Zimmerman, Harrisburg, Pa., originally collected in Florida, $2n = 18$, Bowden (1945a).

Asimina reticulata Chapm.—Florida; 1. col. by Erdman West, Gainesville, Florida, $2n = 18$.

Asimina tetramera Small.—Florida; 1. col. in Florida by Dr. G. A. Zimmerman, Harrisburg, Pa., $2n = 18$.

Asimina pygmaea (Bartr.) Dunal (= *A. angustifolia* A. Gray).—Georgia, Florida, Alabama; 1. col. by R. Hindery and Erdman West, 4 mi. east of Gainesville, Florida, $2n = 18$.

Asimina speciosa Nash.—Flag-Papaw; Florida and Georgia. 1. col. by Erdman West, Gainesville, Florida, $2n = 18$.

ARTABOTRYS R. BR.

Artabotrys odoratissimus R. Br. — Climbing Ylang-Ylang; India to China and the Philippines, and cultivated; 1. col. by Walter R. Lindsay, Canal Zone Experiment Gardens, Summit, Canal Zone, Panama, $2n = 18$; 2. $2n = 18$, E.K.J. in Darlington and Janaki Ammal (1945); 3. $n = 8$, $2n = 16$, Asana and Adatia (1945).

POLYALTHIA BLUME

Polyalthia longifolia (Lam.) Hook. f.—Maraillupai; India and cultivated there as an avenue tree. 1. $n = 9$, $2n = 18$, Asana and Adatia (1945).

CANANGA RUMPH EX HOOK F. & THOMS.

Cananga odorata (Lamb.) Hook. f. & Thoms.—Ylang-Ylang; southern India, Java, and Philippines; cultivated; 1. col. by S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$, Bowden (1945a) and fig. 1 of this paper; 2. col. by Walter R. Lindsay, Canal Zone Experiment Gardens, Summit, Canal Zone, Panama, $2n = 16$.

ANNONA L.

Group Guanabani Safford, Section Euannona Safford.

Annona muricata L.—Sour-Sop; Tropical America and widespread in tropical cultivation. 1. Berlin-Dahlem Botanic Garden, Germany, $2n = 16$, Bowden (1945a); 2. two populations, seed col. by Walter R. Lindsay, Canal Zone Experiment Gardens, Summit, Canal Zone, Panama, $2n = 16$, fig. 2; 3. two populations, seed col. by F. G. Walsingham, Atkins Institution of the Arnold Arboretum, Soledad, Cienfuegos, Cuba, $2n = 16$; 4. $n = 7$, Kumar and Ranadive (1941); 5. $2n = 14$, E.K.J. in Darlington and Janaki Ammal (1945).

Annona montana MacFad. — Jamaica; "Wild Sour-Sop"; 1. Buitenzorg Botanic Garden, Java, $2n = 16$, Bowden (1945a).

Group Attae Safford, Section Atta Mart.

Annona reticulata L.—Custard-Apple; Tropical America; widespread in tropical cultivation; 1. col. by S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$, Bowden (1945a); 2. another population from same source, $2n = 16$; 3. col. by F. G. Walsingham, Atkins Institution of the Arnold Arboretum, Soledad, Cienfuegos, Cuba,

$2n = 16$; 4. pink-fruited variety from Villavicencio, Colombia, South America, through Dr. David Fairchild, Coconut Grove, Florida, $2n = 16$, fig. 3; 5. $n = 7$, Kumar and Ranadive (1941); 6. $n = 7$, $2n = 14$, Asana and Adatia (1945); 7. $2n = 14$, E.K.J. in Darlington and Janaki Ammal (1945).

Annona squamosa L. — Sweet-Sop; Tropical America and widespread in tropical cultivation; 1. from Dr. G. A. Zimmerman, Harrisburg, Pa., $2n = 16$, Bowden (1945a); 2. green-fruited var. col. by S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$, fig. 4; 3. purple-fruited variety col. by S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$; 4. col. by F. G. Walsingham, Atkins Institution of the Arnold Arboretum, Soledad, Cienfuegos, Cuba, $2n = 16$; 5. $n = 7$, Kumar and Ranadive (1941); 6. $n = 7$, $2n = 14$, Asana and Adatia (1945).

Annona Cherimola Mill.—Cherimoya; Andes of Peru and Ecuador; widely cultivated in Sub-tropics; 1. col. by Dr. David Fairchild from large-fruited trees, Colombia, South America, and obtained through S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$; 2. "ssp. Cherimerina" from Venezuela, South America, through S. J. Lunch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$; 3. large-seeded variety from Venezuela, South America, through S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$; 4. col. by J. Samper, Bogota, Colombia, South America, and sent through Dr. David Fairchild, Coconut Grove, Florida, $2n = 16$; 5. Dept. of Botany greenhouse, University of Toronto, Toronto, Ont., $2n = 16$; 6. col. in the interior of Panama by Walter R. Lindsay, Canal Zone Experiment Gardens, Summit, Canal Zone, Panama, $2n = 16$, fig. 5, 6; 7. $n = 7$, Kumar and Ranadive (1941); 8. $2n = 14$, E.K.J. in Darlington and Janaki Ammal (1945).

Annona squamosa × *A. Cherimola.*—Atemoya; cultigen; 1. Page Atemoya, seed from S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$; 2. U.S.D.A. Atemoya, seed from S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 16$.

Group Acutiflorae Safford, Section Phelloxylon Safford.

Annona glabra L.—Pond-Apple; Tropical America, West Africa; used as a grafting stock; 1. from Dr. G. A. Zimmerman, Harrisburg, Pa., $2n = 28$, Bowden (1945a); 2. a form with atypical fruit, from Reasoner Bros. Nurseries, Bradenton, Florida, and through S. J. Lynch, Sub-tropical Experiment Station, Homestead, Florida, $2n = 28$, fig. 7; 3. col. by F. G. Walsingham, Atkins Institution of the Arnold Arboretum, Soledad, Cienfuegos, Cuba, $2n = 28$; 4. $2n = 28$, E.K.J. in Darlington and Janaki Ammal (1945).

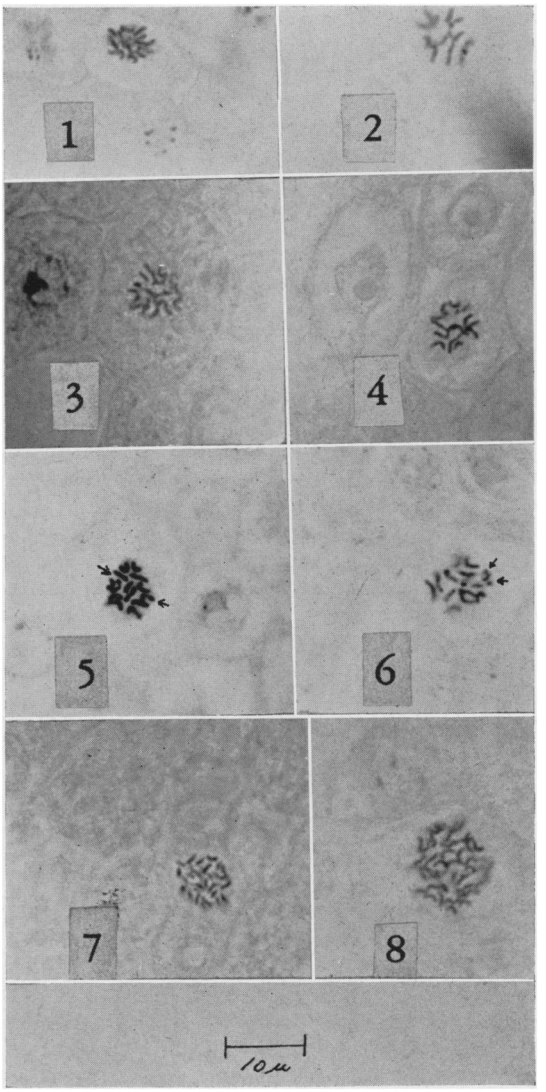

Fig. 1–8. Photomicrographs of mitotic metaphases in periblem cells of root tips.—Fig. 1. *Cananga odorata* (Lamb.) Hook. f. and Thoms., $2n = 16$.—Fig. 2. *Annona muricata* L., $2n = 16$.—Fig. 3. *Annona reticulata* L., $2n = 16$.—Fig. 4. *Annona squamosa* L., $2n = 16$.—Fig. 5. *Annona Cherimola* Mill., $2n = 16$.—Fig. 6. *Annona Cherimola* Mill., $2n = 16$.—Fig. 7.—*Annona glabra* L., $2n = 28$.—Fig. 8. *Rollinia* sp., $2n = 48$. The arrows in fig. 5 and 6 indicate the small chromosomes.

ROLLINIA ST. HIL.

Rollinia sp. (may be *R. orthopetala* A. DC.).—Tropical America; 1. from Medellin, Colombia, South America, and collected by Dr. E. Ruiz Landa, Arboretum Nacional, Medellin, Colombia, South America, sent through Wilson Popenoe and Dr. David Fairchild, $2n = 48$, fig. 8.

Discussion.—Janaki Ammal's counts agree with those of the author for *Artabotrys odoratissimus* and *Annona glabra;* and Asana and Adatia's count for *Polyalthia longifolia* agrees with the data for

other species of Annonaceae. The counts of the following authors do not fit the available data for these species: Asana and Adatia (1945)—*Artabotrys odoratissimus, Annona reticulata,* and *Annona squamosa;* Kumar and Ranadive (1941)—*Annona muricata, Annona reticulata, Annona squamosa, Annona Cherimola;* Janaki Ammal in Darlington and Janaki Ammal (1945)—*Annona muricata, Annona reticulata, Annona Cherimola.* In these cases the above authors have reported one fewer pair of chromosomes in the species complements compared to the author's counts. These discrepancies in counting may have been caused by failure to observe the two small chromosomes which are often obscured by the larger chromosomes.

In table 1, the available data are summarized for chromosome numbers of seventeen species and one inter-specific hybrid in six genera of the Annonaceae. The chromosome numbers listed in table 1 are those which are considered to be valid by the present author. The possibility that other chromosome number races exist cannot be determined until further reports on annonaceous material have been made.

The numbers $2n = 18$ and $2n = 16$ occur in both Old and New World species. The North American species are $2n = 18$ or $x = 9$; while the South American species which have been studied are mainly $2n = 16$ ($x = 8$); however, there is one South American species with $2n = 28$ ($x = 7$ or $x = 8$) and one with $2n = 48$ ($x = 8$). Seven North American species of *Asimina* are $2n = 18$; and, in view of the evidence on hybridization in *Asimina* which has been given by Zimmerman (1941), it appears that this is a natural genus of closely-related species. Fries (1939) listed eight species in the genus *Asimina* Adans.: the seven reported in this paper and *Asimina secundiflora* Shuttlw. Rehder and Dayton (1944) recognized two more species: *Asimina pulchella* (Small) Rehder and Dayton and *Asimina Rugelii* Robinson. Merrill (1945) has discussed the taxonomic problems of some of these shrubby species.

It is obvious that only a very small proportion of the total number of species and genera of the Annonaceae has now been studied cytologically. However, the data indicate that cytological information will be valuable in ascertaining taxonomic and phylogenetic relationships. Hutchinson (1923) classified the ninety-five genera of the Annonaceae. The genera *Asimina, Artabotrys, Polyalthia,* and *Cananga* belong to subfamily Anonoideae, tribe Unoneae, subtribe Xylopineae and series Hexapetalae. The genera *Annona* and *Rollinia* belong to the same subfamily and tribe, but are placed in subtribe Anonineae. The cytological data are in agreement with this classification except that *Cananga odorata* has $2n = 16$ while the other species of subtribe Xylopineae have $2n = 18$; and *Annona glabra* has $2n = 28$ while the five other species of *Annona* have $2n = 16$. *A. glabra* may be a tetraploid in an $x = 7$ series, or a tetraploid from an $x = 8$ series, which has had a rearrangement of chromosome number. The fact that *A. glabra* has a different chromosome number from the other species of *Annona* can be utilized in identifying material. The seedlings grown from seed from a tree with atypical fruit from Reasoner Bros. Nurseries, resembled *A. glabra* seedlings; and the count of $2n = 28$ confirmed the opinion that the material was *A. glabra.*

Darlington and Janaki Ammal (1945) listed five species of Annonaceae in the $x = 7$ series and two species in the $x = 9$ series. The new data in the present paper show that only one species remains in the $x = 7$ series, namely, *Annona glabra.* It will be interesting to learn if $x = 7$ will be found in some of the species which have not yet been investigated. Hutchinson (1926) stated that the Annonaceae were "related to but a more advanced and fixed type than the Magnoliaceae." Whitaker (1933) found diploids and tetraploids with a base number of $x = 19$ in seven species of the Magnoliaceae. He considered that $x = 19$ was an unusual chromosome number. The predominance of $x = 9$ and $x = 8$ in the Annonaceae does not indicate a close relationship with the Magnoliaceae. Darlington and Janaki Ammal (1945) listed data which showed that the Magnoliaceae had $x = 7$ and $x = 19$; Winteraceae, $x = 7$ and $x = 19$; Schisandraceae, $x = 7$; Trochodendraceae, $x = 7$ and $x = 19$; Cercidiphyllaceae, $x = 19$; Annonaceae, $x = 7$ and $x = 9$; and Lauraceae, $x = 7$ and $x = 12$. The data in the present paper show that $x = 8$ and $x = 9$ are the commonest base numbers in the species of Annona-

TABLE 1. *Chromosome numbers in Annonaceae.*

Genus	Geographical distribution	Number of species	Chromosome number ($2n$)
Asimina	Temperate and sub-tropical North America	7	18
Asimina	Eastern North America	1	27 (triploid mutants)
Artabotrys	Old World Tropics	1	18
Polyalthia	India	1	18
Cananga	Old World Tropics	1	16
Annona	Tropical and sub-tropical America	5	16
Annona	Hybrid (cultigen)	1	16
Annona	Tropical America, West Africa	1	28
Rollinia	Tropical America	1	48

ceae which have been investigated cytologically. Nine species belong to the $x = 9$ series, seven species to the $x = 8$ series, and one species belongs to the $x = 7$ series. Until more data on chromosome number, size, and morphology are available for a large number of species, it will be difficult to draw final conclusions regarding the relationship of the cytological data to phylogenetic classification. However, the species of Annonaceae do show distinctive cytological characteristics such as chromosome number, range of chromosome size and fixation image.

Bowden (1948) found five triploids and two hundred diploids in twenty-three seedling populations of the North American Papaw, *Asimina triloba* (L.) Dunal. The other species of Annonaceae have not been investigated on a large enough scale to know whether or not mutants occur in them, but it would be worthwhile to try to find mutants in some of the useful tropical species.

SUMMARY

New chromosome number determinations are listed for twenty-nine populations of thirteen annonaceous species and one inter-specific hybrid. Previously published data are reviewed and the available cytological data are summarized for seventeen species in six genera. In root-tip preparations, $2n = 18$ occurred in seven species of *Asimina* and in one species each of *Artabotrys* and *Polyalthia;* $2n = 16$ in one species of *Cananga* and in five species and one species hybrid of *Annona;* $2n = 28$ in one species of *Annona* and $2n = 48$ in one species of *Rollinia.* Five triploid ($2n = 27$) mutants were found among 200 diploid ($2n = 18$) seedlings of *Asimina triloba.*

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