Chromosome Numbers in the Polemoniaceae

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This family is divided by systematists into two subfamilies. Cobaeoideae is composed of the genera *Cantua*, *Huthia*, and *Cobaea* which are tall shrubs, trees, or vines. The approximately fourteen other (herbaceous or low shrubby) genera comprise the subfamily Polemonioideae. From the standpoint of number of included species the most important of these latter genera are *Gilia*, *Phlox*, *Polemonium*, and *Collomia*. The genera *Phlox* and *Polemonium* are each quite distinct and their species are seldom confused with those of other genera, but the remaining twelve genera of this subfamily apparently do not have taxonomic boundaries of equivalent distinctness. Certain species have been placed in one genus by one author, and in one or more different genera by others. The genus *Gilia* has been extended by some writers to include practically all species of the Polemonioideae outside of the genera *Phlox* and *Polemonium*. This was first pointed out to me by Professor Edgar T. Wherry who is making a taxonomic and geographic study of the Polemoniaceae, and was increasingly emphasized as literature sources and herbarium specimens were consulted and examined.

It has been the purpose of the work herein presented to secure data on all obtainable polemoniaceous species with respect to chromosome numbers, size, and general morphology. Especial attention has been given to securing information of significance from a taxonomic standpoint. Since several factors combine to make the immediate completion of the original program impossible it seems desirable, meanwhile, to present the accumulated data, together with suggested conclusions.

Representatives of nine genera have been available for this study. The genera *Polemonium* and *Phlox*, as noted below, have been subjects of previous cytological work; chromosome counts in several species of these genera not covered by the previous surveys have been made and are included here. Brief reports of chromosome observations have previously been made on single species of three other genera of the family. These are indicated in table 1 by giving the name of the original author opposite the species involved.

Materials

Plants or seeds were obtained from botanists, professional collectors, seed houses, and in some cases by personal collections. The greatest proportion of the material was obtained as seed from commercial houses and, as was perhaps to be expected with plants in a taxonomically confused family, proved in many cases to have been assigned incorrect specific and even incorrect generic names. No material of uncertain identity at the time of its receipt is included in this report unless it was grown to the flowering stage and then checked. Pressed specimens on which the identity of the species concerned may be established have been kept.

Methods

Most of the material has been studied cytologically in smear preparations. Meiotic material has been prepared by Belling's (1926) iron-aceto-carmin method, or by modifications of this. Root tip material has been prepared by a modification of Warmke's (1935) root tip smear method.

Some material was embedded in paraffin and sectioned. Zirkle's (1930) method of dehydrating tissues by use of *n*-butyl alcohol, in the higher series, works well with *Gilia* tissues. Johansen's (1935) tertiary butyl alcohol dehydration method gives excellent preparations; sections do not adhere to the slide as smoothly as with *n*-butyl alcohol, but the time from killing to staining is greatly shortened and cellular details are superb.

Drawings were made with the aid of an Abbe Camera-lucida. Original magnifications of these were approximately $\times 3400$ with three exceptions; Fig. 2 was drawn originally at about $\times 2400$, and Figs. 3 and 4 were made at approximately $\times 3000$. All figures have been reduced one-half in reproduction.

Chromosome Numbers

This section summarizes chromosome counts in the Polemoniaceae as known from the present and past work of the writer and of other workers. Previously unreported counts in 40 different species or well differentiated varieties are given here. About 105 polemoniaceous lines were investigated in the present work; some of these were representatives of the same species secured from different sources; some were lines previously reported upon but were included here to clear up questionable points. The species studied fall into several cytological groups recognizable chiefly upon the basis

of chromosome numbers but also, to some extent, by general chromosome morphology.

Figs. 1-8. Chromosomes in *Gilia* species. 1-4, 7, and 8 from aceto-carmin smears; 5 and 6 from paraffin embedded, sectioned, material. 1. *Gilia rigidula*; metaphase; $n = 9$. 2. *G. rigidula*; metaphase; $2n = 18$. 3. *G. (Ipomopsis) rubra*; anaphase; $2n = 14$. 4. *G. (Ipomopsis) rubra*; metaphase; $n = 7$. 5. *G. millefoliata*; metaphase; $2n = 36$. 6. *G. millefoliata*; II metaphase (complements on both spindles shown); $n = 18$. 7. *G. tricolor*; metaphase; $2n = 18$. 8. *G. tricolor*; metaphase; $n = 9$ (chromosomes separated in drawing).

In the genus *Phlox* 7 is the basic chromosome number for all forms examined both in this and previous studies. Most *Phlox* species are diploids. The exceptions found are low polyploids, three being tetraploids and one seemingly a triploid.

The present work has shown two basic numbers in plants commonly assigned to the genus *Gilia*. These are 7 and 9, the latter number being more frequent. One species of this genus, *G. millefoliata*, is apparently a tetraploid with 36 chromosomes (Figs. 5 and 6), although in an earlier, embryological, study Schnarf (1921) re-

ported 16 pairs of chromosomes in this species. Several genera often included in *Gilia* but probably deserving to be separated from that genus have basic numbers of either 7 or 9. These genera will be discussed in more detail below.

In *Polemonium* previous investigations (Clausen 1931) as well as the present work have revealed only diploid species with 9 pairs of chromosomes.

Figs. 9-19. Chromosomes in species from several genera of the Polemoniaceae. 9, 10, 13, 14, 16, and 17 from aceto-carmin smears; 11, 12, 15, 18, and 19 from paraffin embedded, sectioned, material. 9. *Collomia grandiflora*; metaphase; $2n = 16$. 10. *C. grandiflora*; metaphase; $2n = 32$; tetraploid cell in root tip of diploid plant. 11. *C. coccinea*; metaphase; $2n = 32$. 12. *C. coccinea*; metaphase; $n = 16$. 13. *Hugelja virgata*; metaphase; $2n = 14$. 14. *Cobaea scandens*; metaphase; $n = 26$. 15. *C. scandens*; metaphase; $2n = 52$. 16. *Linanthus dianthiflorus*; metaphase; $2n = 18$. 17. *Polemonium boreale*; metaphase; $2n = 18$. 18. *Cantua buxifolia*; metaphase; $2n = \text{ca. } 54$. 19. *Phlox nivalis*; metaphase; $2n = 14$.

The genus *Collomia* has a basic number of 8, three species having been found with 16 chromosomes, and a fourth possessing 32 somatic chromosomes. In this genus occasional tetraploid root tip cells have been found in diploid species (Fig. 10).

Cobaea has been represented by a single species in the present work. This was observed to have 26 pairs of chromosomes (Figs. 14 and 15). Lawson (1898) in studying the karyokinetic spindles in the P.M.C.'s of this species (*C. scandens*) says "The largest number (of chromosomes) observed in polar view was twelve." It is thus possible that there are both diploid and tetraploid forms of this species, the latter having been encountered in my work.

Cantua also has been represented by only one species here. The exact chromosome number has not yet been ascertained but there are approximately 54 in somatic nuclei (Fig. 18).

The above discussion shows that in the subfamily Polemonioidae there are three basic chromosome numbers, viz., 7, 8, and 9. In the other division of the family, Cobaeoideae, chromosome numbers are much higher with bases of 26, and, perhaps, 27 in the ones investigated.

Table 1 includes a complete list of available chromosome numbers of polemoniaceous plants with the exception of most of the species of *Phlox*, previously reported upon by the writer (Flory 1934). Numbers are given, however, for *P. buckleyi* and for *P. adsurgens*; for the former because they had not previously been observed in the P.M.C.'s of this interesting species; for the latter because the number for authentic material of this species differs from that of the horticultural form sold under that name which had been used in a previous study. The names given for the species worked with in the present study are the checked and corrected ones, which differ in a number of cases from those given at the source of the material. Numbers not reported initially in this paper are followed by the name of the original author.

Chromosome Size and Morphology

Chromosome size depends, to some extent, upon the fixative used. Acetic acid has a swelling effect upon chromatin; most of the acids commonly used in fixing prior to paraffin embedding are more likely to shrink chromatin. Hence in smear preparations the chromosomes of a species may be appreciably longer than in sections from the same species prepared by the paraffin method. For a reliable comparison of chromosome length all mounts upon which measurements have been made should be prepared in as nearly identical manners as possible. The somatic chromosome lengths recorded here have

Table 1. Chromosome numbers in the Polemoniaceae

Species	n	2n	Authors
Polemonioideae			
<i>Collomia coccinea</i> Lehm.	16	32	
<i>C. grandiflora</i> Dougl.	8	16	
<i>C. hetero hylla</i> Hook		16	
<i>C. linearis</i>		16	
<i>Gilia achilleaefolia</i> Benth.	9	18	
<i>G. aggregata</i> (Pursh.) Spreng.	7	14	
<i>G. capitata</i> Dougl. ex Hook		18	Flory, 1934
<i>G. lonoiflora</i> (Torr.) G. Don		14	
<i>G. millefoliata</i> Fisch. & Mey.	16		Schnarf, 1921
<i>G. millefoliata</i> Fisch. & Mey.	18	36	
<i>G. rigidula</i> Benth.	9	18	
<i>G. rubra</i> (L.) Heller	7	14	
<i>G. tricolor</i> Benth.	9	18	
<i>G. tricolor nivalis</i> Hort.	9	18	
<i>Hugelia virgata</i> Benth.		14	
<i>Leptodactylon californicum</i> Hook. & Arn.		18	
<i>Linanthus aureus</i> Greene		18	
<i>L. densiflorus</i> Millik.		18	
<i>L. dianthiflorus</i> Greene		18	
<i>L. dichotomus</i> Benth.		18	
<i>L. parviflorus</i> Greene		18	
<i>Phlox adsurgens</i> Hort.		ca. 21	Flory, 1934
<i>P. adsurgens</i> Torr. ex Gray		14	
<i>P. amplifolia</i> Britton	7		
<i>P. bifida</i> Beck.		14	
<i>P. buckleyi</i> Wherry (P.M.C's)	14		
<i>P. Hoodii</i> Hort.		28	
<i>P. longiflora</i> Hort.		14	
<i>P. nivalis</i> Lodd.		14	
<i>P. paniculata</i> L. hort. vars.			
Ferdinand Cortez	7		
Terre Neuve	7		
Von Hochberg	7		
Widar	7		
<i>Polemonium boreale</i> Adams		18	
<i>P. caeruleum</i>	9	18	Clausen, 1931
<i>P. caeruleum album</i> Hort.		18	
<i>P. c. gracile</i> (Willd.) Brand		18	
<i>P. c. himalayanum</i> Baker	9	18	Clausen, 1931 ; present account
<i>P. c. monstrosum</i> Hort.		18	
<i>P. carneum</i> Gray	9	18	Clausen, 1931
<i>P. confertum</i> Gray	9		
<i>P. filicinum</i> Greene	9	18	Clausen, 1931 ; present account
<i>P. mexicanum</i> Cerv.	9	18	Clausen, 1931 ; present account
<i>P. molle</i> Greene		18	
<i>P. pauciflorum</i> Wats.	9	18	Clausen, 1931
<i>P. pulcherrimum</i> Hook	9	18	Clausen, 1931
<i>P. reptans</i> L.	9	18	Clausen, 1931 ; present account
<i>P. Richardsonii</i> Hort.	9	18	
<i>P. van Bruntiae</i> Britton		18	
Cobaeoideae			
<i>Cantua buxifolia</i> Juss. ex Lam.		ca. 54	
<i>Cobaea scandens</i> Cav.	ca. 12		Lawson, 1898
<i>C. scandens</i> Cav.	26	52	

all been secured from preparations made by the modified Warmke's method.

In Table 2 are indicated the size, approximate point of spindle fiber attachment, and number of satellites observed, of chromosomes of species representing the different cytological groups encountered. In material from some species, especially those with small chromosomes, the spindle fiber constrictions do not show clearly. Where no evidence of a constriction is seen in either smeared or sectioned preparations, the chromosome is said to have a terminal attachment point. There is a possibility that at least some of the chromosomes so designated may actually have attachments other than terminal.

Table 2. Data on somatic chromosomes of representative species in different cytological groups

representative	2n	length in microns		spindle fiber attachment point				no. of satellites observed
		shortest	longest	terminal (or nearly so)	sub-terminal	median	submedian	
1. <i>Cobaea scandens</i>	52	1.	2.5	42	4	6		2+
2. <i>Collomia grandiflora</i>	16	3.5	9.	4	10		2	2
3. <i>Gilia achilleaeifolia</i>	18	4.5	9.		8	4-0	6-10	2
4. <i>Gilia (Ipomopsis) rubra</i>	14	7.5	10.		10		4	4
5. <i>Hugelia virgata</i>	14	5.	7.5		8	2	4	?
6. <i>Linanthus dianthiflorus</i>	18	2.	4.5	2	10		6	2+
7. <i>Phlox bifida</i>	14	5.	9.		10	4		2+
8. <i>Polemonium boreale</i>	18	7.	11.5		2	16		2+

Cytological Groups—Taxonomic Implications

Cobaea and *Cantua*, of the subfamily Cobaeoideae, have many more, and much smaller, chromosomes than have the genera in the Polemonoideae. Also the nuclear-cytoplasmic ratio is much larger here than in other genera of this family. No *Huthia* species have been available for study. If this genus, together with *Cobaea* and *Cantua* really form a natural subdivision of the Polemoniaceae, as is commonly suggested, we would also expect to find many small chromosomes in it. There are certain taxonomic similarities between *Cobaea* and members of the Bignoniaceae, although the 3-celled ovary of this genus is not duplicated in any known representative of the family mentioned. Also *Cantua* and *Fouquieria* show some taxonomic relationship. It may be that when the natural relationships of the genera now placed in the Cobaeoideae are deciphered, these may be found to properly belong elsewhere. It will suffice for the present to say that the cytological picture presented by *Cobaea* and *Cantua* material is decidedly different from that shown by other members of the *Phlox* family.

As indicated above, the genera *Collomia*, *Gilia*, *Hugelia*, *Linanthus*, *Leptodactylon*, *Ipomopsis*, together with about six others, are often collectively grouped and all their representatives called "Gilias". According to Craig's work (1934) *Hugelia* has considerable taxonomic claim to being grouped as a sub-division of the genus *Gilia*. Certain of the other genera, however, do not appear to have close enough taxonomic affinities to be lumped with *Gilia*, and the cytological differences found tend to substantiate the taxonomical relationships.

Thus, members of the genus *Collomia* have 8 pairs of chromosomes (or multiples thereof) and by this number are distinguished cytologically from all other representatives of the family. This and other cytological features (see Table 2 above) support the contention of many taxonomists that Gray made a serious error in combining this genus with the genus *Gilia*.

Gilia achilleaefolia is representative of the true Gilias in taxonomic and in cytological characters. The above table lists the most conspicuous cytological distinctions of this member of the group. *Gilia rigidula* and *G. tricolor* are among the other good species of this group. Figs. 1, 2, 7, and 8 depict the chromosomes of these species.

The species usually listed as *Gilia rubra*, *G. aggregata*, and *G. longiflora* seem to be closely related morphologically. A study of their chromosomes strengthens the view that they are indeed very closely related to each other and are different enough from the true Gilias to be worthy of the generic distinction sometimes accorded them in placing them in the genus *Ipomopsis*, proposed by Michaux as early as 1803.

A representative *Hugelia* species (see Table 1) apparently has only 7 pairs of chromosomes, thus differing from plants which unquestionably belong in *Gilia*. Chromosome numbers then would lend support to the contentions of Bentham (1833) and of Jepson (1925) that *Hugelia* is generically distinct rather than a sub-genus of *Gilia*, as urged recently by Craig, l.c.

The species sometimes placed in the genera *Linanthus* and *Leptodactylon* have 9 pairs of chromosomes. In that respect there is no cytological difference between these and the true Gilias. However these segregates from *Gilia* prove to have measurably smaller chromosomes; the exact figures for representatives of each group may be compared in table 2 above. Hence the cytological evidence tends to substantiate the taxonomical facts which favor the setting apart of these two genera. No cytological differences have yet been observed, however, between *Linanthus* and *Leptodactylon*.

The remaining two cytological groups that may be recognized are those observed in the genera *Phlox* and *Polemonium*. The present study confirms the earlier descriptions of the chromosome pictures observed in these genera by Flory, l.c., and Clausen, l.c.

Drawings of the chromosomes of species representative of the different cytological groups are shown in Figs. 1-19.

Detailed descriptions of the cytological groups are not given here. The chief data bearing on such descriptions are given in Table 2 of the preceding section.

In some plant groups there seems to be no connection between chromosome numbers and taxonomic relationships. An example of this is furnished by the work of Gershoy (1934) on *Viola*. In many cases, however, there does appear to be a definite correlation between chromosome number, morphology, etc., and the other affinities of related forms. Where taxonomical relationships are best understood in the Polemoniaceae (as in the genus *Phlox*), closely related plants are found to have chromosome complements identical in number of elements and similar in appearance. It has seemed reasonable to assume then, in the discussion of this family, that chromosome characters such as number per complement, size, and structure, are valuable diagnostic characters of the species and genera concerned.

Summary

Previously unreported counts on 40 well differentiated species or varieties of the Polemoniaceae are given. Included with these is a general account of all chromosome numbers reported for species of this family to date. This is followed by a summary of chromosome size, chromosome morphology, and the different cytological groups observed. There is a discussion on the taxonomic grouping implied by the cytological facts.

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Literature Cited

- Belling, J. 1926. The iron-acetocarmine method of fixing and staining chromosomes. Biol. Bull. 50: 160-161.
Bentham, George. 1833. Bot. Reg. 19: sub. t. 1622.

- Clausen, J. 1931. Genetic studies in *Polemonium*. III. Preliminary account on the cytology of species and specific hybrids. *Hereditas* 15: 62-66.
- Craig, T. 1934. A revision of the subgenus *Hugelina* of the genus *Gilia* (Polemoniaceae). *Bull. Torr. Bot. Club.* 61: 385-396; 411-428.
- Flory, W. S., Jr. 1934. A cytological study on the genus *Phlox*. *Cytologia* 6: 1-18.
- Gershoy, A. 1934. Studies in North American violets. III. Chromosome numbers and species characters. *Ver. Agr. Exp. Sta. Bull.* 367.
- Jepson, W. L. 1925. A manual of the flowering plants of California. Associated Student Store, Berkeley. pp. 782, 792.
- Johansen, D. A. 1935. Dehydration and infiltration. *Science*, N. S. 82: 253-254.
- Lawson, A. A. 1898. Some observations on the development of the karyokinetic spindle in the pollen-mother-cells of *Cobaea scandens* Cav. *Proc. Calif. Acad. Sci. Ser. III*, 1: 170-188.
- Schnarf, K. 1921. Kleine Beiträge zur Entwicklungsgeschichte der Angiospermen. I. *Gilia millefoliata* Fisch. et Mey. *Oesterr. Bot. Zeitschr.* 70: 153-158.
- Warmke, H. E. 1935. A permanent root tip smear method. *Stain Tech.* 10: 101-103.
- Zirkle, C. 1930. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. *Science* 71: 103-104.
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