CHROMOSOME STUDIES OF PHLOX

JAMES R. MEYER
The Blandy Experimental Farm, University of Virginia

Received October 19, 1943

PHLOX is a genus of 50 species, all of which are native to North America except one, which is in Siberia. In the taxonomic treatment of the species of eastern North America, Phlox has been divided into the Subulatae (*Phlox nivalis* Lodd., *P. subulata* L., and *P. bifida* Beck.), the Divaricatae (*P. divaricata* L., *P. pilosa* L., *P. floridana* Benth., and *P. amoena* Sims.), the Drummondianae (*P. Drummondii* Hook.), and the Ovatae (*P. stolonifera* Sims., *P. ovata* L., *P. carolina* L., *P. glaberrima* L., *P. amplifolia* Britton., *P. paniculata* L., and *P. buckleyi* Wherry (WHERRY 1930, in SMALL 1933).

The chromosomes of Phlox are relatively large and comparatively few in number. Therefore the genus is favorable for intensive chromosome studies. This work is an attempt to throw some light on the phylogenetic relationships of the species by means of chromosome studies.

Interspecific and intraspecific variation in gross morphological characters is very striking, often clear cut, and common in wild as well as in cultivated forms. This variation includes differences in the color, size, and shape of the corolla, in the time of blooming and profuseness of flowers, in the growth rate and habit of the plant, and in the presence or absence of functional sex organs. The question arises as to whether or not there are interspecific differences in chromosome structure and morphology which would be comparable to the morphological differences between species. These studies show that interspecific chromosome differences do exist and that chromosome differences occur within some species, just as do gross morphological differences such as flower color, shape, size, plant growth habit, leaf color, evergreen foliage, and many others.

MATERIALS AND METHODS

The plants used were obtained from various wild habitats, from nurseries, from the U. S. DEPARTMENT OF AGRICULTURE, from DR. J. P. KELLEY, and one colchicine-induced tetraploid of *Phlox Drummondii* from seed of material previously treated by MR. J. HERBERT TAYLOR. They were grown at The Blandy Experimental Farm, of the UNIVERSITY OF VIRGINIA, at Boyce, Virginia. The wild plant collections represent numerous samples from various parts of the range of each species.

Mitotic chromosome counts and idiograms were made from slides made by the colchicine-Feulgen leaf smear method. This involves the use of a colchicine pretreatment, fixation in Semmen's Carnoy, HCl hydrolysis, staining in decolorized basic fuchsin, smearing in 45 percent acetic acid, and making the slide permanent (MEYER 1943). Anthers were fixed in acetic alcohol (3:1), hydrolyzed in HCl, stained and smeared in iron-propiocarmine, and made permanent for meiotic studies.

Idiograms were made at a magnification of 3520×; meiotic drawings were made at 2640×. These magnifications have been reduced to 2350× for the idiograms and 1760× for the meiotic drawings.

TABLE 1

The 2n chromosome numbers of the plants investigated.

NUMBER OF PLANTS EXAMINED	NAME	2N NUMBER
7	*Phlox amoena*	14
1	*P. amplifolia*	14
2	*P. Arendsii* vars. Emma & Louise	14
1	*P. bifida*	14
1	*P. buckleyi*	28
1	*P. carolina heterophylla*	14
1	*P. carolina heterophylla*	14 & 2 ff.
9	*P. divaricata*	14
1	*P. divaricata*	14 & 1 f.
7	*P. Drummondii* vars. astylis, cuspidata, light yellow funnel, pink, Rosy Morn, streak, Plant D14	14
1	*P. Drummondii* var. pink (4X induced)	28
2	*P. glaberrima*	14 & 1–3 ff.
13	*P. nivalis* var. Sir Guilford and others	14
3	*P. nivalis*	14 & 2–4 ff.
6	*P. ovata* var. pulchra and others	14
126	*P. paniculata* plants and vars.*	14
42	*P. paniculata* vars.*	14 & 1–6 ff.
3	*P. paniculata* vars. Hodur, Mrs. Jenkins (Independence), Mrs. Jenner	14 & 7–8 ff.
2	*P. paniculata* vars. Commander and Mme. Louise Abbema	14 & 10 ff.
3	*P. pilosa*	14
2	*P. procumbens* var. *coerulea* and another plant	14
1	*P. stellaria*	14
1	*P. stolonifera*	14
18	*P. subulata* vars.*	14
1	*P. subulata* var. cuspidata	14 & 0–6 ff.
1	*P. subulata* plant 36	14 & 0–13 ff.
4	*P. subulata* plants 23, 115, 194, 385	28
3	*P. suffruticosa* vars. Miss Verboom, Princess Ingrid, Rosalinda	14
1	*P. suffruticosa* var. Rosalinda**	14 & 1 f.
1	*P. suffruticosa* var. Miss Lingard	21
2	*P. nivalis* (plant 34) × *P. subulata* (plant 37) Plants 25-1, 25-2	14
4	*P. nivalis* (plant 33D) × *P. subulata* (plant 23) Plants 26-1, 26-2, 26-3, 26-4	28

* Plants of *P. paniculata* belonging to the following varieties were examined, and all of them had 2n = 14 with no fragments: Africa, Aida, Albion (two sources), Amazon, Annie Cook, Arnold Turner, Asia, Aspasie, Athis, Atlas (two sources), Avista, Bacchante, Baron Ritte, Baron von Heckerer, B. Compte, Beacon (three sources), Beauty, Beauty of Framingham, Betty Lou, Border Gem, Bouquet Fleuri, Bridesmaid (two sources), Brunette, Cameron, Caran d'Arch, Caroline Vandenburg, Cendrillon, Cheswick, Colorado, Columbia, Commander in Chief, Comus, Compte, Count Zeppelin (two sources), Cyclone, Daily Sketch, Danton, Debs, Diana, Dr. Baker, Dr. Charcot, Dr. Paul Reclus, Dwarf White, Eclaireur, Eclipse, Electra, Esclarmonde, Etta's Choice, Europe, Evelyn, Feuerbrand (two sources), Flora Horning, Frederick Passey, Gen. von Heutz, Geo. B. Dorr, Glenwood, Hervor, Hoffangner Stark, Imperator, Improved Meteor, Isabey (two sources), James Bennett, Josephine Gerbeaux, Jules Jany, Jules Sandeau (two sources), Katherine, La Vague, Leo Schlageter, Le Soleil, L'Esperance, Matapoisette Seedling, Meteor,

The ratio obtained by dividing the length of the short arm of a mitotic chromosome by the total length of the chromosome is used in this paper as a means of comparing chromosomes which may be in the same plant or in different plants. This ratio gives the proportionate part that the short arm length is of the total length.

Chromatid area was found by means of the formula $A=\pi Dl$, and chromatid volume by the formula $V=\pi r^2 l$. The chromatid radius = r, chromatid diameter = D, and chromatid length = l.

RESULTS AND DISCUSSION

Chromosome number

The 2n chromosome numbers of the forms which were examined are given in table 1. In addition to one induced tetraploid and six species hybrids, the chromosomes of 264 forms were counted. Of these, 201 (76.1 percent) are diploid (2n = 14), 57 (21.6 percent) are diploid and have from one to 13 additional small centric fragments (2n = 14 and 1–13 fragments), 5 (1.9 percent) are tetraploid (2n = 28), and one (0.4 percent) is triploid (2n = 21).

Most of the plants which were investigated had the diploid somatic number of 14 chromosomes. *Phlox suffruticosa* Miss Lingard was the only triploid found. Because of its low frequency, tetraploidy seems to be a rather unimportant means of specific differentiation among the Phlox species of eastern North America, although the five forms found were all from wild plant collections.

The chromosomes of the small, centric type deserve special attention, since they are especially common in Phlox. For convenience, these chromosomes will be referred to as "fragments."

More than one-fifth of all the plants of Phlox examined during these investigations had one or more small fragment chromosomes in addition to the normal diploid complement of fourteen "major" chromosomes. These fragments were found to vary greatly in number.

Michael Buchner, Mistral, Mme. Paul Dutrie, Mme. Prosper Langier, Mrs. Chas. Dorr, Mrs. Cook, Mrs. Ethel Pritchard, Mrs. Milly von Hoboken (two sources), Mrs. Scholton, Mrs. W. F. Schmieske, Mrs. W. von Beuningen, Nana Coerulea, New Bird, Painted Lady, Pastel Pink, Peach Blow, Prof. Virchow, Regulus, Reich V. H. Hochburg, Rheinlander, Richard Strauss, Richard Wallace, Rijnstroom, Rose Gem, Rosetta, Rubis, Saison Lierval, Siebold, Sommerkleid, Special French, Stella's Choice, Sylphide, The Pearl, Tigress, Venus, Verin Morton's Seedling, Victor, Viking, W. C. Eagan (two sources), Widar, and Plants 151, 157, and 380.

The following varieties of *P. paniculata* had 2n = 14 and 1–6 fragments: Argon, Belvidere, Brogniart, Czarina, Defiance, Eclaireur,** Edmund Rostand, Enchantress (two sources), Flora J. Reidy (two sources), Fiancee, Frau G. v. Lassburg, Gladstone, Graf von Ungerer, Helene Vascaresco, Henri Reynault, Inspecteur Elpel, La Candeur, Le Cygne, Leo Schlageter,** Lothair, Mary Louise, Mia Ruys, Miss Elphick, Mme. Cordunet, Mrs. Arnold Turner, Mrs. Dwyer, Mrs. Ingalls, Mrs. Scholton,** Obergartner Wittig, Pearl, Pecheur d'Island, Philas, Rheinlander,** Rosenburg, Rose Pearl, R. P. Struthers, Special French,** Tapis Blanc, Wellesley, Wolfgang von Goethe.

The following varieties of *P. subulata* were examined and found to have 2n = 14: alba, atropurpurea, Blue Hills, Emerald Cushion, lilacina, Lilakonigen, Maischnee, Newberry Seedling, rosea, Samson, Sensation, Vivid, Wilsonii, and plants 0, 37, 190, 193, and 195.

** A variety containing both plants with and plants without fragments.

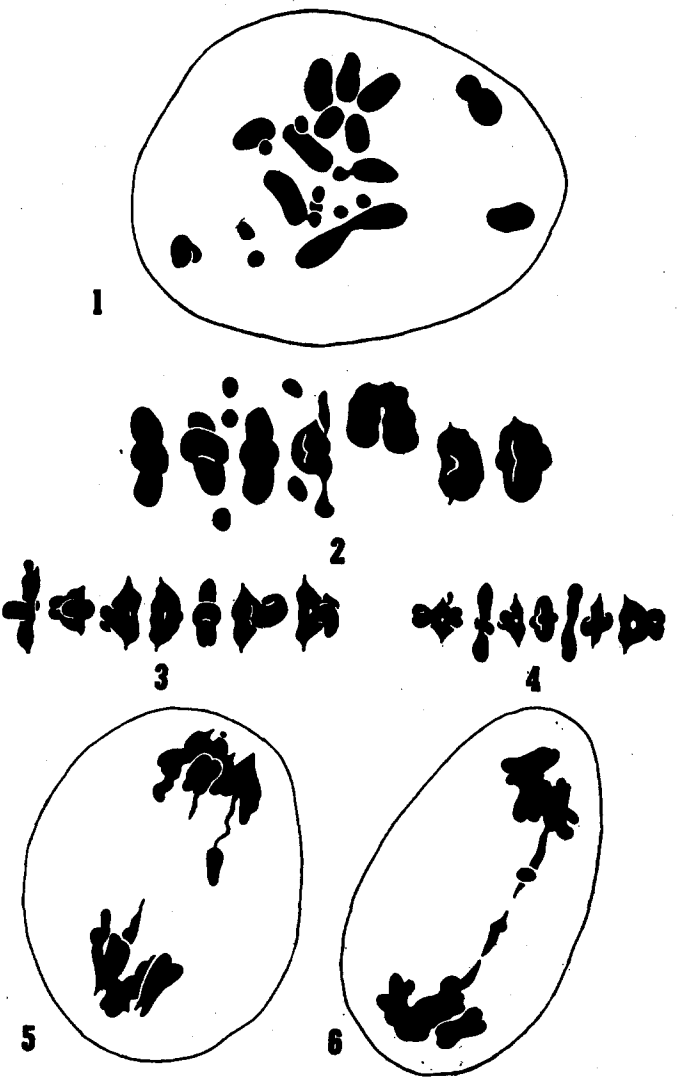

FIGURE 1.—*Phlox paniculata* Commander. Metaphase I with one bivalent, 12 univalents, and ten fragments. Two fragments are associated with major chromosomes, the remaining eight are unpaired. 1760×.

FIGURE 2.—*P. paniculata* Frau G. von Lassburg. Metaphase I with seven bivalents (one bivalent not properly oriented) and seven fragments. Two fragments are held by chiasmata to a major bivalent. 1760×.

FIGURE 3.—*P. paniculata* Mrs. Ingalls. Metaphase I with seven bivalents, a fragment associated with the first bivalent. 1760×.

FIGURE 4.—*P. subulata* cuspidata (35). Metaphase I with seven bivalents, a fragment associated with the first bivalent. 1760×.

FIGURE 5.—*P. paniculata* Mrs. Ingalls. Anaphase I with two broken inversion bridges. 1760×.

FIGURE 6.—*P. paniculata* Mrs. Ingalls. Anaphase I with one breaking inversion bridge and a fragment. 1760×.

In five cultivated varieties of *Phlox paniculata* (Eclaireur, Leo Schlageter, Mrs. Scholton, Rheinlander, and Special French) where two plants from different sources were examined, it was found that one plant of each variety had no fragments, while the other plant had one or more fragments. The same was true in the case of *P. suffruticosa* Rosalinda (table 1). These varieties are all propagated vegetatively and thus each variety represents a clone.

TABLE 2

Fragment length as a proportionate part of the average major chromosome length.

PLANT NO.	NAME	CHROMOSOMES		FRAGMENTS		AV. FRAG. / AV. CHROM.
		NO.	AVERAGE LENGTH	NO.	AVERAGE LENGTH	
167	*P. divaricata*	14	10.1	1	3.0	.30
161	*P. glaberrima*	14	16.3	3	1.1	.07
33B	*P. nivalis*	14	13.4	2	6.3	.47
186	*P. nivalis*	14	10.0	4	2.7	.27
187	*P. nivalis*	14	15.4	3	3.8	.25
129	*P. paniculata* Flora J. Reidy	14	14.6	3	5.5	.38
152	*P. paniculata* Mrs. Jenner	14	23.9	8	3.6	.15
35	*P. subulata*	14	15.5	6	2.1	.14
36	*P. subulata*	14	12.5	1	3.0	.24
36	*P. subulata*	14	13.4	11	2.9	.22

In *Phlox subulata* plant 36 the fragment number varied from 0 to 13 per cell. One of the branch tips examined had from seven to nine fragments, another had from eight to thirteen, and four other branches varied somewhat, but were mostly of one-fragment tissue. In one of these tips consisting mainly of one-fragment tissue, two adjacent metaphases were seen; one metaphase had two fragments and the other had none. It is very probable that these were sister cells and that during the previous division the undivided fragment was included in one of the two resulting nuclei, thereby giving one "daughter" cell with two fragments and the other cell with none at all.

Thus different plants of the same variety, or clone, or different cells in the same plant may have different numbers of fragments, or some of the plants or cells may even contain no fragments. It is clear that the observed percentage of plants with fragments is a minimum.

The size of the fragments in Phlox varies considerably. The fragments of *P. glaberrima* plant 161 are .07, or 7 percent of the average major chromosome length in this plant. In *P. nivalis* plant 33B the fragments are 47 percent of the average major chromosome length. Fragment lengths vary between these extremes in other plants (see table 2).

During meiosis the fragments in Phlox are usually not paired (fig. 1, 2) but may be associated by chiasmata to the centromere region of bivalents (fig. 2, 3, 4), or to univalent major chromosomes (fig. 1). In all the cases where a fragment is associated with a bivalent, it can be seen that the fragment is united by a lateral chiasma very near a centromere of the bivalent. The near-

ness of pairing to the centromere suggests that the fragments are simple duplications of the centromere region; these have arisen by the loss of the distal portions of the major chromosomes.

Fragments have been found by the author in more than 20 percent of the Phlox plants examined and in seven of the eighteen species studied. The variation in fragment number among plants of the same cultivated variety, or clone, and among the cells within certain plants is due to a lack of synchronization in the orientation and division of the fragments with respect to the timing of these processes in the major chromosomes. Although the fragments within a plant seem to be equal in size, fragment size varies greatly from plant to plant.

By what mechanism could fragments of different sizes be formed so frequently in so many different species? The loss of the distal portion of the extra chromosome of a trisomic would be a possible means of fragment formation. However, it is very improbable that this occurs commonly, since no trisomics in Phlox are known by this writer, with the exception of one plant of *P. procumbens* which FLORY (1934) found to be partially trisomic and partially diploid.

The fragments in Phlox must have originated by a process which can occur frequently. Otherwise the fragments would not be seen so frequently, they would not vary so much in size, and they would not occur in so many different species as they do.

Inversions are very common in Phlox. Of 20 plants examined, 14 (70 percent) formed inversion bridges during meiosis. Figure 5 shows the remains of two inversion bridges, and figure 6 shows the remains of another. Note that two of these inversion bridges have broken so that a large acentric fragment is formed from the central portion of each bridge. It seems possible that the centric fragments of Phlox may arise as the ends of broken inversion bridges. The size of the centric fragments would vary inversely as the size of the acentric fragment which forms from the bridge breakage. Since fragments are known to orient and divide non-synchronously in mitosis, it is easy to see how this could happen during meiosis, thus forming gametes with a full set of chromosomes and a new fragment. If this is the method by which fragments usually originate in Phlox, then they arise at anaphase of meiosis, as they do in Uvularia (BELLING 1925), and not at meiotic prophase as in Tradescantia (DARLINGTON 1929).

In the tomato (LESLEY and LESLEY 1929) the fragments were about half the usual chromosome size. When one was present in a diploid, that plant differed less from the true diploid than did the corresponding primary trisomic. These fragments were genetically active, but not so active as whole chromosomes. In Phlox there were no gross morphological differences noted between plants of the same horticultural variety when those plants differed in the number of fragments they contained. The plant which has from 0 to 13 fragments per cell seems to have nearly identical flowers and stems and leaves in all parts. Since plants in the same variety, or cells in the same plant, may vary in fragment number (or lose all their fragments) without producing noticeable

morphological changes, and since the cells and plants may survive without the fragments, it seems reasonable to conclude that, in general, the fragments in Phlox are either nearly or entirely genetically inert. No evidence was found for identifying the fragments with heterochromatin.

To summarize, most of the forms of Phlox which were examined have the diploid number of 14 chromosomes. Polyploidy has not been important, with few exceptions, in the differentiation of the Phlox species of eastern North America. The occurrence of fragments in the various species seems to have no relationship to Phlox taxonomy.

Chromosome size

During the course of these studies, idiograms were made of many of the plants examined. These idiograms show the differences in chromosome size and morphology within a complement, but the length of the chromosomes in two idiograms is not comparable, even though these two idiograms were made from two chromosome plates in the same plant. The reason for this is that the chromosomes in the various cells are shortened (and thickened) to different degrees during the colchicine pretreatment. This differential shortening is caused by varying amounts of effective colchicine pretreatment in the different cells. The colchicine will be more highly concentrated in the outer cells than in the central cells of a region. Likewise, some nuclei will divide before others and thus be subject to the shortening effect of the colchicine for a longer time.

Since the lengths of the colchicine-treated chromosomes are not comparable, the volumes and surface areas have been computed from chromatid length and width data. The volumes of chromosomes have been previously used as a means of comparison by many workers, but this writer knows of no attempt to use chromosome surface area as a basis for comparison.

It is well known that chromosomes contain, or perhaps they consist of, coiled chromonemata. In mitotic chromosomes these are called minor or somatic or standard coils by the different workers. In Phlox these coils are often seen in late mitotic prophase chromosomes on slides made by the colchicine-Feulgen leaf smear technique; they have also been seen in the anaphase chromosomes of tapetal tissue. In both of these cases the mitotic coils resembled the coils in the spring of a window-shade rod. Thus, since the coils of chromatin (Feulgen+) material seem to be mostly confined to the chromatid surface in this material, the thought occurred that chromatid area might be a good means for comparing chromosome size in different plants.

The relative areas and volumes of some Phlox chromosomes are given in tables 3 and 4. In regard to variation within a species, table 3 shows little variation of mitotic chromosome size in the plants of *Phlox nivalis*, while *P. Drummondii* Rosy Morn has chromosomes about one-half as large as those of *P. Drummondii* pink (2×). The average chromosome of *P. paniculata* Jules Sandeau is about three and one-half times the size of the average chromosome of *P. paniculata* Victor.

Chromosome size is compared in eight diploids and three tetraploids of

TABLE 3

Relative surface areas and volumes of entire complements and average Phlox chromosomes.†

PLANT NO.	NAME	2n	TOTAL AREA	AV. AREA	TOTAL VOLUME	AV. VOLUME
168	*P. buckleyi*	28	2789.0	99.54	1254.0	44.78
D14	*P. Drummondii*	14	1178.0	84.45	706.8	50.49
—	*P. Drummondii* astylis	14	960.5	68.44	432.1	30.79
—	*P. Drummondii* cuspidata	14	1222.0	87.10	672.2	48.01
—	*P. Drummondii* light yellow funnel	14	1177.0	83.29	529.8	37.91
—	*P. Drummondii* pink (2X)	14	1455.0	103.3	831.4	59.41
—	*P. Drummondii* pink (4X)	28	2699.0	96.40	1754.0	62.65
—	*P. Drummondii* Rosy Morn	14	778.5	55.62	292.0	20.85
—	*P. Drummondii* streak	14	1046.0	74.80	627.7	44.88
33B	*P. nivalis*	*14	1180.0	84.20	576.2	41.16
33D	*P. nivalis*	14	954.8	67.95	501.3	35.92
34	*P. nivalis*	14	1132.0	80.69	679.7	48.42
110	*P. nivalis*	14	971.2	69.28	509.9	36.38
122	*P. nivalis*	14	862.8	61.58	431.4	30.81
173	*P. nivalis*	14	1155.0	82.47	722.1	51.54
175	*P. nivalis*	14	1132.0	80.87	622.7	44.48
185	*P. nivalis*	14	1169.0	83.33	759.7	54.16
186	*P. nivalis*	*14	964.2	68.85	530.4	37.87
187	*P. nivalis*	*14	1349.0	96.36	674.5	48.39
189	*P. nivalis*	14	895.2	63.89	514.7	36.74
191	*P. nivalis*	14	1045.0	74.66	470.2	33.59
192	*P. nivalis*	14	957.0	68.31	574.2	41.00
128	*P. paniculata* Aida	14	1462.0	104.1	840.7	59.84
129	*P. paniculata* Flora J. Reidy	*14	1798.0	128.4	1258.0	89.91
135	*P. paniculata* Compte	14	1294.0	92.49	744.2	53.19
137	*P. paniculata* Jules Sandeau	14	4025.0	287.7	1861.0	133.0
142	*P. paniculata* Beacon	14	2995.0	213.9	1985.0	141.8
151	*P. paniculata* (wild)	14	1782.0	127.8	826.2	59.10
152	*P. paniculata* Mrs. Jenner	*14	1629.0	116.3	631.0	45.10
156	*P. paniculata* Europe	14	1969.0	140.6	1034.0	73.43
157	*P. paniculata* (wild)	14	2083.0	148.8	1614.0	115.4
200	*P. paniculata* Aspasie	14	1360.0	96.76	748.0	53.4
201	*P. paniculata* Victor	14	1111.0	79.23	555.6	39.59
202	*P. paniculata* Mme. Prosper Langier	14	1409.0	100.9	669.4	47.92
203	*P. paniculata* Eclipse	14	1510.0	107.4	755.0	53.73
204	*P. paniculata* Caroline Vandenburg	14	1203.0	85.98	571.4	40.83
380	*P. paniculata* (wild)	14	2089.0	149.2	1462.0	104.1
0	*P. subulata*	14	1167.0	83.45	466.8	33.38
23	*P. subulata*	28	1450.0	51.84	543.8	19.45
35	*P. subulata* cuspidata	*14	1026.0	73.05	384.7	27.39
36	*P. subulata*	*14	1041.0	74.63	494.4	35.3
36	*P. subulata*	*14	1118.0	80.00	531.0	37.99
37	*P. subulata*	14	1141.0	81.5	541.8	38.56
190	*P. subulata*	14	1194.0	84.96	477.7	33.98

TABLE 3—*Continued*

PLANT NO.	NAME	2n	TOTAL AREA	AV. AREA	TOTAL VOLUME	AV. VOLUME
193	*P. subulata*	14	1173.0	83.58	556.9	39.69
194	*P. subulata*	28	1398.0	50.01	559.4	20.01
195	*P. subulata*	14	673.9	48.11	320.0	22.85
385	*P. subulata*	28	2271.0	81.11	1249.0	44.61
25-1	*P. nivalis*×*P. subulata*	14	1196.0	85.33	418.5	29.86
25-2	*P. nivalis*×*P. subulata*	14	996.1	71.06	323.8	23.10
26-1	*P. nivalis*×*P. subulata*	28	2451.0	87.66	919.4	32.88
26-2	*P. nivalis*×*P. subulata*	28	2770.0	99.28	1385.0	49.65
26-3	*P. nivalis*×*P. subulata*	28	2408.0	85.98	1144.0	40.71
26-4	*P. nivalis*×*P. subulata*	28	2240.0	80.0	671.9	23.87

* These plants have 2n=14 & 1 or more ff.

† These areas and volumes are computed from measurements in mm at 3500×.

Phlox subulata. The average chromosome areas of the diploids vary from 48 to 85 units, thus the chromosomes of no diploid are double the size of those of another. The relative sizes of the chromosomes of the three tetraploids vary as 50, 52, and 81. It may be seen that the diploids vary from 48 to 85 units while the tetraploids vary from 50 to 81 (table 3). The slight increase in the variance of the diploids over that of the tetraploids could easily be due to the fact that the chromosomes of eight diploids, but only three tetraploids, were measured. More measurements might show that the tetraploids, and not the diploids, vary most in average chromosome size.

The chromosomes of the induced tetraploid of *Phlox Drummondii* pink are very little, if any, smaller than the chromosomes of the diploid form (table 3); thus the 28 chromosomes in the tetraploid have about twice the area and volume of the 14 chromosomes in the diploid. This is similar to the situation in regard to the diploid and tetraploid forms of *Ranunculus Ficaria* (LARTER 1932) and is different from *Primula kewensis*, where the total chromosome volume per nucleus is the same in the diploid as in the tetraploid form (FARMER and DIGBY 1914).

Two diploid hybrids were produced by crossing a diploid *Phlox nivalis* used as ♀ with a diploid *P. subulata*. Hybrid 25-2 (fig. 7, 8) has much larger chromosomes at metaphase I than has its sister hybrid 25-1 (fig. 9). However, the mitotic chromosomes of hybrid 25-2 are smaller than those of hybrid 25-1 (table 3).

The size difference in the meiotic chromosomes of these hybrids is probably due to the genotypic control of chromosome size, as was found in the triploid *Tradescantia brevicaulis* (DARLINGTON 1929), in *Melandrium album* (BRESLAWETZ 1929), and in *Lolium perenne* seedlings (THOMAS 1936).

Hybrid 25-2 has larger meiotic, but smaller mitotic chromosomes than has its sister hybrid 25-1. Likewise hybrid 26-3 has larger meiotic chromosomes than have hybrids 26-1, 26-2, and 26-4, but it has smaller mitotic chromosomes

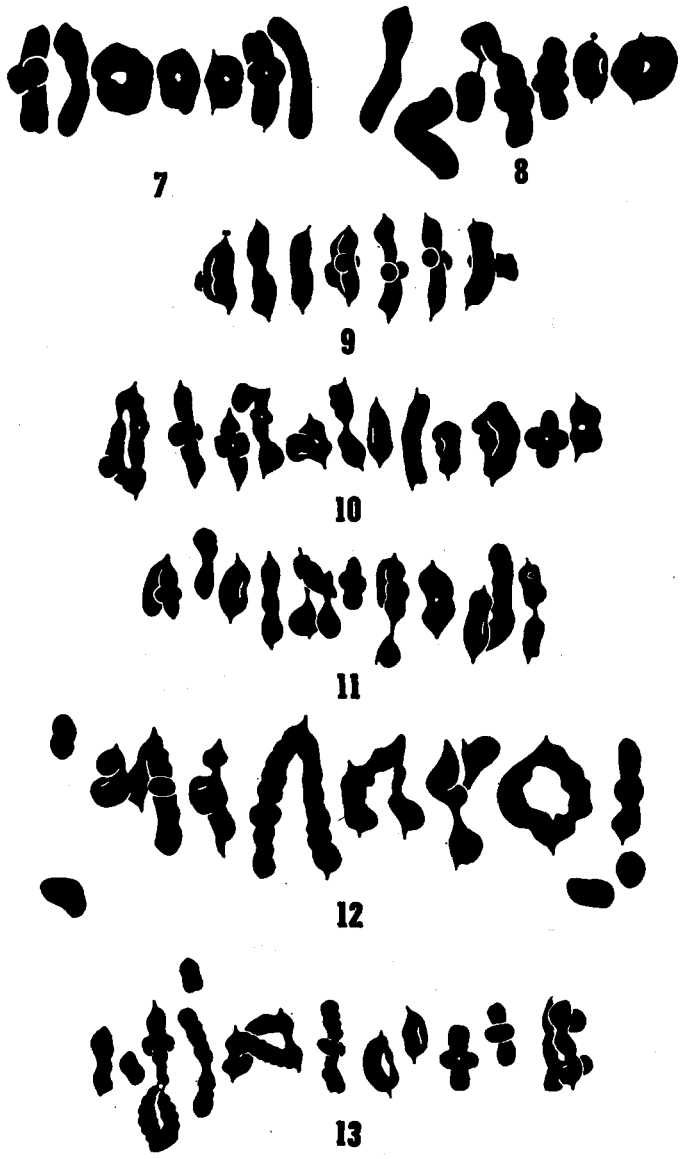

FIGURE 7.—*P. nivalis*×*P. subulata* hybrid (25-2). Metaphase I with seven bivalents. 1760×.

FIGURE 8.—*P. nivalis*×*P. subulata* hybrid (25-2). Metaphase I with one quadrivalent and five bivalents. 1760×.

FIGURE 9.—*P. nivalis*×*P. subulata* hybrid (25-1). Metaphase I with seven bivalents. These chromosomes are smaller than those of hybrid 25-2 (fig. 7, 8). 1760×.

FIGURE 10.—*P. nivalis*×*P. subulata* hybrid (26-1). Metaphase I with two quadrivalents and ten bivalents. 1760×.

FIGURE 11.—*P. nivalis*×*P. subulata* hybrid (26-2). Metaphase I with four quadrivalents and six bivalents. 1760×.

FIGURE 12.—*P. nivalis*×*P. subulata* hybrid (26-3). Metaphase I with five quadrivalents, two bivalents, and four univalents. These chromosomes are larger than those of hybrids 26-1, 26-2, and 26-4 (fig. 10, 11, 13). 1760×.

FIGURE 13.—*P. nivalis*×*P. subulata* hybrid (26-4). Metaphase I with three quadrivalents, seven bivalents, and two univalents. 1760×.

TABLE 4

*Range of variation in average chromosome area and volume.**

SPECIES	NUMBER OF PLANTS	AVERAGE AREA	AVERAGE VOLUME
Subulatae			
Phlox bifida	1	75	37
P. nivalis	13	62–97	28–54
P. stellaria	1	102	43
P. subulata	11	48–85	19–45
Divaricatae			
P. amoena	3	72–116	38–77
P. divaricata	6	63–120	33–69
P. pilosa	2	79–99	33–50
Drummondianae			
P. Drummondii	8	56–103	21–63
Ovatae			
P. buckleyi	1	100	45
P. carolina heterophylla	1	129	81
P. glaberrima	1	154	115
P. ovata	5	84–124	48–88
P. paniculata	15	79–288	40–142
P. stolonifera	1	74	37
P. suffruticosa	3	94–200	52–110

* These areas and volumes are computed from measurements in mm at 3500×.

than hybrid 26-2. These seeming discrepancies are perfectly understandable, since the meiotic chromosomes have major coils, the gyres of which may vary in number, diameter, and distance from each other. The genotype could determine whether the gyres would be large and rather far apart or of less diameter and closer together at metaphase I; the first condition would give meiotic chromosomes of relatively large size, as in the hybrids 25-2 and 26-3.

Among the Subulatae (*Phlox bifida*, *P. nivalis*, *P. stellaria*, and *P. subulata*) the mitotic chromosomes vary no more in size between species than they do within species. The same holds true for the Divaricatae (*P. divaricata*, *P. pilosa* and *P. amoena*) (see table 4). The Drummondianae include only *P. Drummondii*. In the Ovatae (*P. buckleyi*, *P. paniculata*, *P. glaberrima*, *P. suffruticosa*, *P. carolina heterophylla*, *P. stolonifera*, *and P. ovata*) *P. stolonifera* has the smallest chromosomes of those measured, while *P. paniculata* has the largest chromosomes and the greatest variation in average size.

Chromosome morphology

The one chromosome characteristic which almost all Phlox species have in common is the diploid number 14. The chromosomes differ considerably in morphology in different plants or in varieties within the same species. For instance, the ratio of the SAT-chromosome in *P. subulata* was found to vary from

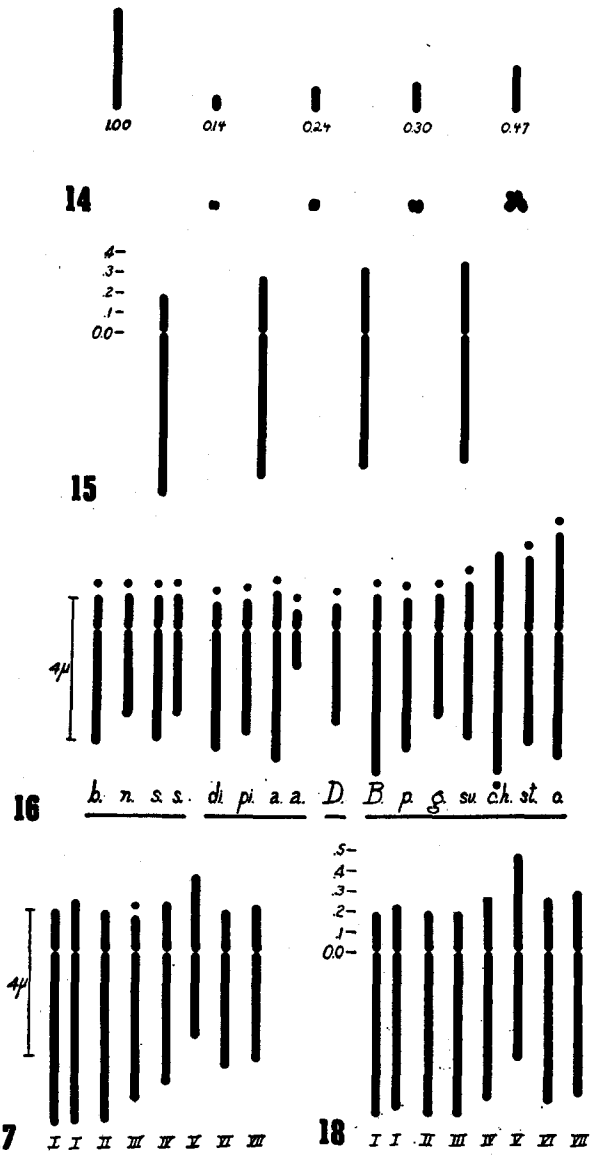

FIGURE 14.—Relative lengths of fragments as compared with the average major chromosome length. In the top the 1.00 diagram represents the length of an average chromosome. The second diagram is 0.14 (or 14 percent) of the average chromosome length; fragments of this relative length were found in *P. subulata* plant 35. The diagram of the fragment which is .24 (or 24 per cent) of the average major chromosome length is from *P. subulata* plant 36. The fragment with a .30 (30 percent) average major chromosome length is from *P. divaricata* plant 167, the .47 (47 percent) fragment is from *P. nivalis* 33B. Drawings under the diagrams show the respective fragments at 2350×.

FIGURE 15.—*P. subulata*. Idiogram showing the range of variation in arm ratio of the SAT-chromosome of different plants, from .19, in *P. subulata* cuspidata (plant 35) to .36 in plant 194.

.19 to .36 (see fig. 15). This intraspecific variation makes it difficult to make generalizations that will apply to a species.

Chromosome length was of no use for comparing the chromosomes of different plants, so use was made of an arm length ratio. The ratio obtained by dividing the short arm length by the total chromosome length was used as a means of comparison; this ratio expresses the proportionate part that the short arm is of the entire chromosome length. In each case, when an idiogram was made and studied, the chromosomes were arranged as to total length (the longest first and the shortest last), the ratio for each chromosome was found, and then the chromosomes which were judged to be similar enough in regard to total length and arm ratio were paired. After this was done, the pairs of chromosomes (say of chromosome I, the longest pair) were often found to vary in ratio in the different plants within a species. Usually, however, most of the longest pairs of chromosomes had very nearly the same ratio within a species. This was also true when chromosome II, the SAT-chromosome, and chromosome VII were compared in different plants and varieties within a species. These four chromosomes (I, II, SAT-, and VII) were distinctive enough so that they could usually be picked out of a complement, and average ratios are recorded for them in the various species (table 5). The other three kinds of the seven pairs of chromosomes were not distinctive in either total length or arm ratio, so no data from the study of them is included in the table. In some cases, as the SAT-chromosome of *Phlox subulata*, there seemed to be two main chromosome types.

Since the SAT-chromosome is the most distinctive and the one which is least likely to be mistaken for another, the species are arranged in table 5 and in figures 16 and 19 to 22 according to the SAT-chromosome arm ratio.

In the Subulatae it may be seen (table 5) that *Phlox nivalis* and *P. subulata*, which are very much alike morphologically, also have chromosomes with very similar arm ratios. Except for chromosome VII, the chromosomes of *P. bifida* have ratios quite unlike those of *P. nivalis* and *P. subulata; P. bifida* differs considerably in appearance from *P. nivalis* and *P. subulata*. On the other hand, *P. stellaria*, which is similar in appearance to *P. subulata*, has chromosomes with different ratios.

In the Divaricatae, *Phlox divaricata* and *P. amoena* have similar ratios for chromosomes I, II, and the SAT-chromosome. The SAT-chromosome of *P.*

FIGURE 16.—Idiograms of SAT-chromosomes of various species. These idiograms were made by plotting actual average lengths for the different species. These idiograms are of little use because the various chromosomes are so different in size; this was at least partially due to the colchicine pretreatment. In the Subulatae, b.=*Phlox bifida*, n.=*P. nivalis*, and s.=*P. subulata*. In the Divaricatae, di.=*P. divaricata*, pi.=*P. pilosa*, and a.=*P. amoena*. In the Drummondianae, D.=*P. Drummondii*. In the Ovatae, B.=*P. buckleyi*, p.=*P. paniculata*, g.=*P. glaberrima*, su.=*P. suffruticosa*, c.h.=*P. carolina heterophylla*, st.=*P. stolonifera*, and o.=*P. ovata*.

FIGURE 17.—*P. buckleyi*. Idiogram of the seven kinds of chromosomes in the complement. Actual average lengths were used. There are two types of chromosome I.

FIGURE 18.—*P. buckleyi*. Idiogram of the seven kinds of chromosomes in the complement. The chromosomes have all been given the same length for convenience in comparing their ratios.

TABLE 5

Ratio of short arm length to total length in Phlox chromosomes.

SPECIES	I	II	SAT	VII
Subulatae				
P. bifida	.24	.33	.22	.31
P. nivalis	.32	.24	.28	.33
P. subulata	.33	.21	.28 & .33	.33
P. stellaria	.31	.12	?	.38
Divaricatae				
P. divaricata	.31	.22	.19	.23
P. pilosa	.215	.34	.23	.285
P. amoena	.36	.24	.23	.36
Drummondianae				
P. Drummondii	.23	.18	.22	.16
Ovatae				
P. buckleyi	.19 & .23	.195	.20	.29
P. paniculata	.38	.23	.25	.33
P. glaberrima	.37	?	.29	.30
P. suffruticosa	.37	.23	.30	.26
P. carolina heterophylla	.35	.24	.35	.27
P. stolonifera	.39	.25	.39	.37
P. ovata	.42	.21	.42	.32

pilosa also has a ratio similar to those of the other two species, but its chromosomes I and II are quite different, and the ratio of chromosome VII is different in all three of the species. The similarity of the arm ratios of *P. bifida* of the Subulatae and of *P. pilosa* of the Divaricatae should not go unnoticed; the plants of these two species are similar in appearance, and so perhaps they should be classified in the same sub-genus.

Only *P. Drummondii* is represented from the Drummondianae. Since the ratios of the chromosomes are so variable in the different plants and races, these ratios may not have much significance. However, all the chromosomes of *P. Drummondii* seem to have unusually short second arms.

The chromosomes of *P. buckleyi* (of the Ovatae) are idiogrammed in figures 17 and 18. In figure 17 the actual lengths were idiogrammed, while in figure 18 all the chromosomes were made the same length to facilitate a comparison of their ratios. In the plant which was examined, the 28 chromosomes fell into seven different total length and arm ratio groups, but the four chromosomes of group I were of two pairs with somewhat different ratios. The size differences in chromosomes I to VII can be seen in figure 17, while the ratio differences are more easily seen in figure 18. The chromosomes ranged from 4 to 6μ in length and from .19 to .29 in arm ratio, except chromosome V which had an arm ratio of about .47. This was the highest arm ratio found in any Phlox chromosome.

WHERRY (1930) regards *Phlox buckleyi* as being more closely related to *P. ovata* than to any other form, and FLORY (1934) thinks *P. buckleyi* may be an autotetraploid form of *P. ovata*. A glance at figures 19 and 21 shows that the ratios of chromosome I and of the SAT-chromosome are very different in these two species. Furthermore, the .47 ratio of chromosome V of *P. buckleyi* is totally unlike the ratio of any *P. ovata* chromosome. Therefore, *P. buckleyi* is probably not a form of *P. ovata*.

Notice the similarity between chromosomes I and II and the SAT-chromosome of *P. buckleyi* and *P. Drummondii* (fig. 19–21). The writer thinks that *P. buckleyi* is more closely related to *P. Drummondii* than to other members of the taxonomic group Ovatae. Some evidence from attempts to make species crosses substantiates this view. Crosses were made both ways between *P. buckleyi* and (1) *P. stolonifera*, (2) *P. ovata*, and (3) *P. Drummondii*. Only some of the crosses of *P. buckleyi* upon *P. Drummondii* were successful, and some seeds resulted which did not germinate. This cross was successfully made during two seasons. Of course, it may be that the *P. buckleyi* plant which was examined resulted from the combination of certain reciprocal translocations and the duplication of *P. ovata* chromosomes, and that the cross on *P. Drummondii* was successful because this latter species has short styles, and thus the foreign pollen tubes would have a shorter distance to go in *P. Drummondii*. If these possibilities prove to be the true state of affairs, then the similarity between the chromosomes of *P. buckleyi* and *P. Drummondii* and the "crossability" of the two species are coincidental and of no phylogenetic importance.

The SAT-chromosome of Phlox is usually one of intermediate length, and it often has a subterminal centromere. The satellite was always found on the short arm of the SAT-chromosome, except in *P. carolina heterophylla* and the horticultural hybrid *P. procumbens*, where it is on the long arm. Chromosome I, the longest pair, is also the SAT-chromosome in *P. stolonifera* and *P. ovata;* it has a sub-median centromere and bears the satellite on its shorter arm (see fig. 16, 21).

It may be seen that all the species of the Ovatae with the exception of *P. buckleyi* (*P. paniculata, P. glaberrima, P. suffruticosa, P. carolina heterophylla, P. stolonifera*, and *P. ovata*) have chromosomes which are very similar or grade into one another (fig. 19–22).

The dissimilarity of the chromosome arm length ratios of two unlike species of Phlox seems to indicate that the chromosomes of these two species are differentiated from each other by rather large, or by many small, structural changes. On the other hand, the similarity of the chromosome arm length ratios of two closely related species indicates that the chromosomes of these two species are differentiated by relatively few, or by no, small structural changes. In fact, two closely related species may have almost identical ratios; perhaps their chromosomes are not structurally differentiated.

The comparison of chromosome arm length ratios probably supports, or at least does not conflict with, the taxonomic division of the genus Phlox into the four sub-divisions, with two exceptions. These data suggest that *Phlox*

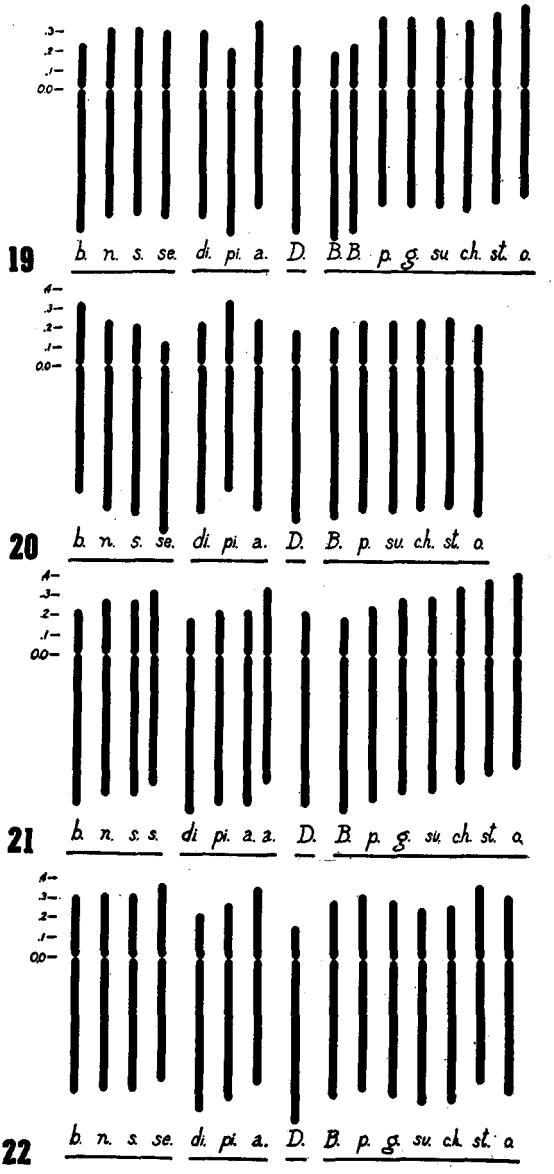

FIGURE 19.—Idiograms of chromosome I for the various species. The chromosomes have all been given the same length for convenience in comparing their ratios. The Subulatae includes *P. bifida* (b.), *P. nivalis* (n.), *P. subulata* (s.), and *P. stellaria* (se.). The Divaricatae includes *P. divaricata* (di.), *P. pilosa* (pi.), and *P. amoena* (a.). The Drummondianae has *P. Drummondii* (D.). The Ovatae includes *P. buckleyi* (B.), *P. paniculata* (p.), *P. glaberrima* (g.), *P. suffruticosa* (su.), *P. carolina heterophylla* (c.h.), *P. stolonifera* (st.), and *P. ovata* (o.).

FIGURE 20.—Chromosome II is compared in the various species, as in figure 19.

FIGURE 21.—The SAT-chromosome is compared in the various species, as in figure 19.

FIGURE 22.—Chromosome VII is compared in the various species, as in figure 19.

bifida and *P. pilosa* may be more closely related than the taxonomic grouping indicates; the same is true of *P. buckleyi* and *P. Drummondii.*

The idiograms in the various species of Phlox bear a general resemblance to one another, but show characteristic minor differences. The diploid complement of *Ranunculus Ficaria* consists of eight different morphological types of chromosomes. The chromosomes of *R. bulbosus* and *R. sardosus* correspond exactly in regard to the position of their centromeres and relative length with those of *R. Ficaria* (LARTER 1932). This situation exists between the chromosomes of *Phlox nivalis* and *P. subulata.* LARTER found that other Ranunculus species differed from the above three in the position of the centromere in one chromosome pair. *R. ophioglossifolius* stands alone in the genus in that it has its satellites attached to the long arm of a subterminally constricted pair. This is very similar to the occurrence of the satellite on the long arm of a chromosome of *Phlox carolina heterophylla* and in the horticultural hybrid *P. procumbens* (plant 29). *P. carolina* was probably a parent of the *P. procumbens* plant which was examined during these investigations.

SUMMARY

The intraspecific variability in both the wild and the cultivated forms of Phlox is very striking. This variation includes differences in the color, size, and shape of the corolla, in the time of blooming and profuseness of flowers, in the growth rate and habit of the plant, and in the presence or absence of functional sex organs.

Of the 264 forms of wild and cultivated Phlox studied, 201 (76.1 percent) are diploid ($2n=14$), 57 (21.6 percent) are diploid with from one to thirteen additional small centric chromosomes ($2n=14$ and 1–13 fragments), five (1.9 percent) are tetraploids found wild ($2n=28$), and one (0.4 percent) is triploid ($2n=21$). Two diploid and four tetraploid species hybrids and an induced tetraploid were also studied.

The number of fragments often varies among the plants of a clone or the cells of a plant.

The fragments in different plants vary from 7 percent to 47 percent of the average major chromosome length.

The presence or absence of fragments does not appear to influence either plant morphology or cell viability. Thus the fragments in Phlox do not seem to be genetically important. No cytological evidence was obtained for identifying them with heterochromatin.

It is suggested that the fragments in Phlox perhaps originate by the breaking of inversion bridges during meiosis.

Phlox suffruticosa Miss Lingard was the only triploid found and has $2n=21$.

Tetraploidy, except in *Phlox buckleyi* and a few others of the 50 known species, seems to have been relatively unimportant in the differentiation of Phlox species.

The average surface areas and average volumes of Phlox chromosomes were compared. Within a species there may be but little variation of mitotic chromo-

some size, or some plants may have chromosomes twice or even three and one half times as large as those of other plants in the same species.

In *Phlox subulata* the diploids and tetraploids were found to have the same range in chromosome size. An induced tetraploid of *P. Drummondii* had chromosomes as large as those of the diploid parent.

One seedling may have larger metaphase I chromosomes and smaller mitotic chromosomes than a sister seedling. This was found in two sets of hybrid seedlings.

In Phlox the SAT-chromosome is usually one of intermediate length with a sub-terminal centromere, the satellite being on the short arm. However, in *P. stolonifera* and *P. ovata* chromosome I is also the SAT-chromosome, and in *P. carolina heterophylla* and the horticultural hybrid *P. procumbens* the satellite is borne on the long arm of the SAT-chromosome.

Considerable variation in the arm length ratio may occur for a chromosome in different plants within a species, as the SAT-chromosome of *Phlox subulata* which varies in arm ratio from .19 to .36.

The chromosomes of *Phlox nivalis* and *P. subulata* are very similar; other species have quite different arm length ratios.

The comparison of chromosome arm length ratios probably supports, or at least does not conflict with, the taxonomic division of the genus Phlox into the Subulatae, the Divaricatae, the Drummondianae, and the Ovatae, with two possible exceptions. *Phlox bifida* and *P. pilosa* may be more closely related than the taxonomic grouping indicates; the same may be said of *P. buckleyi* and *P. Drummondii*.

The writer wishes to express his indebtedness to DR. ORLAND E. WHITE, Director of The Blandy Experimental Farm, for the use of laboratory facilities and materials and for his guidance. He also wishes to thank DR. LADLEY HUSTED for his helpful criticism.

LITERATURE CITED

BELLING, J., 1925 The origin of chromosomal mutations in Uvularia. J. Genet. **15**: 245–266.

BRESLAWETZ, L., 1929 Zytologische Studien über *Melandrium album* L. Planta **7**: 444–460.

DARLINGTON, C. D., 1929 Chromosome behaviour and structural hybridity in the Tradescantiae. J. Genet. **21**: 207–286.

1937 Recent advances in cytology. Sec. Ed. Philadelphia: Blakiston.

FARMER, J. B., and L. DIGBY, 1914 On the dimensions of the chromosomes considered in relation to phylogeny. Phil. Trans. Roy. Soc. London **205**: 1–26.

FLORY, W. S., 1934 A cytological study on the genus Phlox. Cytologia **6**: 1–18.

LARTER, L. N. H., 1932 Chromosome variation and behaviour in Ranunculus L. J. Genet. **26**: 255–283.

LESLEY, J. W., and M. M. LESLEY, 1929 Chromosome fragmentation and mutation in tomato. Genetics **14**: 321–336.

MEYER, J. R., 1943 Colchicine-Feulgen leaf smears. Stain Tech. **18**: 53–56.

SMALL, J. K., 1933 Manual of the Southeastern Flora. New York: The author.

THOMAS, P. T., 1936 Genotypic control of chromosome size. Nature **138**: 402.

WHERRY, E. T., 1930 A long lost Phlox. J. Wash. Acad. Sci. **20**: 25–38.