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IN
AGRICULTURAL SCIENCES

Vol. 2, No. 1, pp. 1-46, pls. 1-12

December 4, 1913

STUDIES IN JUGLANS I

STUDY OF A NEW FORM OF *JUGLANS CALIFORNICA*
WATSON

BY

ERNEST B. BABCOCK

UNIVERSITY OF CALIFORNIA PRESS
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STUDIES IN JUGLANS I

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WATSON

BY
ERNEST B. BABCOCK

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STUDY OF A NEW FORM OF *JUGLANS CALIFORNICA* WATSON

I. HISTORY AND DESCRIPTION

In the autumn of 1900 D. C. Disher, of Garden Grove, California, according to his own account, gathered about two thousand nuts from a certain California black walnut tree that grew near Yorba, in Santa Ana Cañon, but which has since been destroyed. These nuts were planted in the spring of 1901 in order to raise seedlings upon which to graft the English walnut for orchard planting. Among the seedlings about twenty appeared from the first to be distinct from the rest, which resembled the parent tree. Of these twenty only two trees remain where they were first transplanted from seed-bed to nursery row, the others having been given away or destroyed. Of these two only one produces both male and female flowers and bears nuts; the other always produces staminate catkins in abundance but no pistillate flowers. For this reason the first mentioned individual has already been described¹ as "the original fertile tree," but it would have been more exact to have referred to it as Disher's fertile tree, inasmuch as some of the other original specimens above mentioned have been located and are known to bear nuts also. The writer has seen seven of these distributed trees, and material from an eighth; in leaf and bark characters as well as in general appearance they resemble Disher's trees. Three of these are growing at the experimental garden of N. B. Pierce in Santa Ana, three are located in the town of Garden Grove, one is on the Leffingwell Ranch in East Whittier, and one is at the United States Plant Introduction Gardens in Chico, California. One of Pierce's trees is shown in plate 13, figure 16.

The discoverer of these trees wished to preserve them because they are so strikingly different in their leaf characters and in general habit from ordinary California black walnut. They possess no special economic value, being less robust than other walnuts and more restricted in their range of adaptability to adverse

¹ Babcock, E. B., in Jepson, *The Silva of California*, Mem. Univ. Calif. 11 (1910), pp. 50-54.

soil conditions. But their structural characteristics alone are sufficient to excite the interest of the student of plants, especially of one interested in problems of heredity and evolution.

Perhaps the most interesting thing about these trees is their resemblance to oak trees. In mass effect they resemble small-leaved oaks more than walnuts. This is mostly due to the small size of the leaves and to their color, which is a darker or duller shade of green than that of California black walnut leaves. These features, associated with the fact, noted by Disher, that the parent tree stood close beside a coast live oak tree (*Quercus agrifolia* Née), are sufficient to account for the view, held by a number of persons, that this new form originated through hybridization between walnut and oak.

As the seeds were planted in 1901, these trees are now twelve years old. The two retained by Mr. Disher were left in the nursery, which was set out to commercial varieties of walnut (*Juglans regia*) later on by the owner of the farm. He has allowed the trees to stand unmolested, except for trimming up low-hanging branches. Now they have attained a height of twenty-five feet and have a spread of branches about twenty feet in diameter. Seen among the broad-leaved English walnuts, these two trees present a distinct appearance with their many slender branchlets and their sparse foliage. In early spring and late autumn or early winter the contrast is even greater, because these trees and other specimens of the new form resemble southern California black walnuts in the brevity of their dormant period. They leaf out very early in spring and some leaves persist until February. The English walnuts, however, come into leaf from April to June, according to the variety, and by November are once more leafless.

Other distinctive vegetative characters are well shown in plate 1, figure 1. There is a marked tendency to dichotomous branching. This is conspicuous in the left-hand tree in the picture and is noticeable in the other individual. There is also a tendency to form bunches of leaves at the ends of the branchlets. This is apparent in both trees. The appearance of the bark on the trunks of Disher's original trees is also distinct from that of *Juglans californica*. While that of the latter in trees of the

same age as Disher's is usually rough, the bark on these trees is rather smooth.

The left-hand tree in plate 1, figure 1, always produces both male and female flowers and frequently bears abnormal bisexual flowers also. But the other tree produces only staminate catkins, and the photograph shows in what profusion they are borne. A very few pistillate flowers have been seen on this tree, but it has never been known to bear fertile seeds. That sterility in this individual is associated with greater vigor in wood production is shown in the above figure. The leaves on some specimens of the new form are highly variable. The common type of leaf, however, is one with a terminal leaflet one or two inches long and two smaller lateral leaflets, as shown in plate 2, figure 2, *a*. Sometimes both lateral leaflets are missing and occasionally only one is present, as shown in plate 2, figures 2, *b* and *c*, and in plate 3, figures 4 and 5. Plate 2, figure 2, is approximately natural size. In order to appreciate the remarkable difference in leaf characters between the new form and *Juglans californica* note the leaves in plate 7, figure 11.

The frequent occurrence of ascidia (pitchers) on the leaves of the Leffingwell "original" tree gives further evidence of the tendency toward extreme variation. Not being aware that ascidia have been reported previously in the genus *Juglans*, I give plate 2, figure 3 a photograph of four leaves bearing ascidia and one normal leaf.

The nuts borne on Disher's original fertile tree resemble those of southern California black walnuts in external appearance of the husk (cf. plate 2, figure 2). Their average size is much smaller. The immature fruits are also similar, except that many of them are ridged or grooved as in plate 3, figure 4, *a*, and figure 5, *a*, while some depart widely in appearance from the typical young fruit of *Juglans californica*. Such a specimen is shown in plate 3, figure 5, *b*. The smooth area surrounding the stigma was proportionately large, and tapering arms extended towards the base. Surrounding the stigma were several small protuberances, apparently remnants of anthers. The occurrence of bisexual flowers on this tree has been mentioned. When the dry husk is removed from the mature nuts of this tree, they are

seldom found to be divided into nearly equal parts by a deep suture but there are sometimes outer indications of tripartite inner structure. Such marks are shown in plate 4, figure 6, *l*. The occurrence of tricotyly is frequent but many tripartite nuts contain no embryos. Such a nut containing an embryo is shown in plate 4, figure 6, *k*. In dicotyledonous nuts from this tree the cotyledons are reduced, sometimes so much so as to be barely distinguishable (cf. plate 4, figures *g, h, i, j*).

RECURRENCE OF THE NEW FORM

In the autumn of 1907, when the writer first visited Garden Grove to examine the original trees, he was shown about a dozen two-year-old seedlings, all of which closely resembled the original trees. These were found scattered through the nursery of about nine thousand budded English walnut trees. The seedling roots were grown from nuts collected in the autumn of 1904, partly from wild trees in Brea Cañon and partly from a row of *Juglans californica* trees growing in Garden Grove. The seeds had been mixed so that there was no way of locating the tree or trees that gave rise to the new form. Mr. Disher's interest in the unusual appearance of these seedlings again prompted him to leave them unharmed, so that the writer was able to secure a fairly good photograph of one that stood at the end of a row (cf. plate 5, figure 7). The contrast between this little tree and its luxuriant neighbors was certainly striking. The slender branches and ovate leaflets are distinctive. Some leaves are so placed that the large terminal leaflet and two small lateral leaflets are clearly shown. One of the seedlings differed from all the rest in having two pairs of lateral leaflets. Nearly all the leaves on the tree were of this type (cf. plate 5, figure 8). Since that time the writer has seen similar leaves on other seedlings, most of whose leaves were three-parted, as well as leaves intermediate between the two. In fact, the great amount of variation in the leaves of the several individuals which we class as the "new form" is one of the most interesting things connected with it (cf. plates 2, 3, 5, and 11).

In 1909 William Tyler, son-in-law of Mr. Disher, reported to the writer that he had found a few specimens of the aberrant

form among a large number of seedlings of the Garden Grove black walnuts. This was the third appearance of the form in question.

In the autumn of 1910, the writer arranged to have the nuts from the various individual black walnut trees of the row in Garden Grove gathered separately so that they might be planted separately the following spring. The nuts were so gathered, but unfortunately became mixed while in storage. Thus, although about thirty specimens of the new form appeared, they were scattered through the nursery and could be traced to no particular tree or trees. This was the fourth appearance of the new form.

In the autumn of 1911 other parties secured many of the nuts from the Garden Grove trees and although a few aberrant seedlings appeared in Tyler's seed beds, he was not certain which tree produced them. This makes the fifth appearance of the form.

In 1912 the writer had the product of twenty-one of the trees in the Garden Grove row gathered separately. Among the sprouted seedlings of one tree six aberrant seedlings have already appeared, making the sixth appearance. An additional appearance of the new form has been reported. In this case a single seedling appeared among those grown from a mixed lot of southern California black walnut seeds from trees growing wild in Santa Monica Cañon. The seeds were gathered in 1910, so that the tree is now three years old. It resembles Disher's original trees.

From the foregoing account it is evident that the new form has originated in at least three different localities. In two of these, Santa Ana and Santa Monica cañons, the trees were in the wild condition when the nuts were collected. The Garden Grove trees comprise a boundary line planting between two farms. The new form is not reported to occur in the wild and probably does not so occur. However, it would no doubt thrive in the more favorable areas now occupied by wild walnuts in southern California. Seedlings of Disher's original trees have been observed to suffer more from excess of moisture than from drouth.

Walnut breeders and nurserymen have propagated the form by means of grafting. Frank A. Leib, of San José, has a

young tree grown from a cion obtained from N. B. Pierce, of Santa Ana, and grafted on a hybrid walnut root of the "royal" type. The tree has made rather remarkable growth. The Gardena Agricultural High School has obtained seeds of Disher's original tree at Garden Grove in order to grow seedlings for instructional purposes. But there is general confusion as to the true nature of the form and it has been distributed under misleading names. In view of these facts it was deemed advisable to record a name indicating its natural relationship preliminary to the publication of this paper.²

Now, if this form had been first described from specimens collected in the wild, there is no doubt that it would have been named a distinct species. Without endeavoring to solve the problem of its origin the botanist would have felt justified in thus naming it by its absolutely distinct foliage alone. But we do not *know* that it could long exist under wild conditions and, as will be shown later, the seedlings of the original trees do *not* come uniformly true to type. Instead of so doing, usually some of them resemble *J. californica*. Hence, the writer feels justified in calling it a variety of the species from which it has sprung, no matter by what process. The following is a description of Disher's original fertile tree already published³ with two or three minor changes based upon data in the possession of the writer.

NEW VARIETY

JUGLANS CALIFORNICA VAR. QUERCINA BABCOCK

Tree 20 ft. or more high. Bark aromatic and strongly walnut-scented. Branchlets hollow, chambered with pithy plates. Twigs, bud-scales, and young leaves granular-pubescent. Buds few-sealed, axillary or superposed. Leaves 1 to 3 inches long, alternate, exstipulate, mostly compound with three leaflets; terminal leaflet two or three times as long as lateral leaflets and ranging from $\frac{1}{2}$ to 2 inches in length, in form varying from broadly ovate through oval to elliptical or oblong, truncate or emarginate at the apex, margin serrate or almost entire; lateral leaflets placed opposite or scattered, with petiolules or sessile, sometimes one or both lacking; petiole equal to, shorter or longer than, the terminal leaflet. Plant monoecious, occasionally with hermaphrodite

² Babcock, 'A New Variety of *Juglans californica* Wats.' *Science*, n. s., XXXVIII, 968, p. 89.

³ Babcock, Mem. Univ. Calif. II (1910), p. 54.

flowers. Staminate flowers in lateral catkins from wood of the preceding year; calyx adnate to the inconspicuous dark-red bract, irregular, consisting of three larger and one or two smaller lobes and an inner whorl of 4 to 6 smaller distinct sepals, one or two of which sometimes show stamen characters; stamens sepaloïd, 10 to 13, with 1 to 4 of the central ones abortive; filaments free, very short; anthers variable, the pollen sacs unequal, especially in the outermost stamens, connective not bifid at the top. Pistillate catkins 3 to 6-flowered, terminating branchlets of the same season's growth; calyx irregularly 4 to 7-lobed, adherent to the inferior 1-celled ovary; the latter often with 1 or 2 longitudinal grooves or ridges, rarely with 2 or 3 fleshy bracts near the base, occasionally with abortive anthers; styles 3 or 4, short, united toward the base or free, stigmatic along the inside, the fringed surfaces forming a rosette. Fruit similar in appearance to that of *Juglans californica* Wats., but smaller and more variable as to form and internal structure of the nut; the seed also much smaller; cotyledons much reduced, not convoluted (cf. plate 1, figure 1, plate 2, figures 2 and 3, plate 3, figures 4 and 5, plate 4, figure 6, *g, h, i, j, k, l*, plate 5, figures 7 and 8, plate 6, figure 9, plate 11, figures 17 and 18).

DIFFERENCES BETWEEN THE NEW FORM AND THE SPECIES TYPE

J. californica

Leaves, 6 to 13 inches long, compound with 11-19 leaflets.

Terminal leaflet shorter than, equal to, or longer than the lateral leaflets and ranging from 1½ to 4 inches long, oblong lanceolate, serrate.

Staminate flowers. Calyx irregularly 3 to 6-lobed; stamens 20 to 26, connective bifid at the apex.

Pistillate flowers. Calyx 4-lobed; styles 2.

Fruit globose, ¾ to 1 inch in diameter; cotyledons prominent, much convoluted.

J. californica var. *quercina*

Leaves 1 to 3 inches long, mostly compound with 3 leaflets, rarely with 5, sometimes simple.

Terminal leaflet two or three times as long as lateral leaflets and ranging from ½ to 2 inches in length, in form varying from broadly ovate through oval to elliptical or oblong, truncate or emarginate at the apex, margin serrate or almost entire.

Staminate flowers. Calyx adnate to the inconspicuous dark red bract, irregular with 3 larger and 2 smaller lobes and inner whorls of 4-6 smaller distinct sepals; stamens sepaloïd, 10 to 13, anthers variable, connective not bifid at apex.

Pistillate flowers. Calyx irregularly 4 to 7-lobed; styles 3 or 4, short, the fringed surfaces forming a rosette.

Fruit smaller and more variable as to form and internal structure; seed much smaller, cotyledons much reduced, not convoluted.

SUMMARY

1. A new form of walnut has appeared on seven separate occasions among seedlings of at least three different trees of *Juglans californica* Wats.

2. This form is sufficiently distinct from all other walnuts to justify its recognition as a new species. But in all but one of the germination tests of seeds from the original trees, some seeds have produced plants resembling the species type in leaf characters. Moreover, the form is exceedingly variable. These facts are good reasons for describing the new form as a variety of *J. californica*.

II. ORIGIN OF THE NEW FORM—HYPOTHESES,
OBSERVATIONS AND EXPERIMENTS

In studying the nature and origin of this new form of walnut, three working hypotheses have been retained, and two other hypotheses have been eliminated so far as my experimental work is concerned. The latter hypotheses will be discussed briefly before passing to the fuller consideration of the working hypotheses and the investigations connected therewith.

One of the first possibilities suggested was that the new form may be a "reversion to an ancestral type." The species is long-lived and stump-sprouts freely. A few generations might extend back to a time when a now extinct form existed in the same area. It is a well-known fact that our present American species interbreed freely when growing near each other, and also breed with *Juglans regia* under similar conditions. Hence it is conceivable that such an extinct form might have interbred with *J. californica*. According to Mendelian principles, some of the progeny would be heterozygous for certain characters, which might be the distinguishing characters of the new form. Such heterozygous progeny, under favorable conditions, would continue to produce both parent forms and more heterozygous individuals. It is conceivable, then, that the few trees thus far known to exist, which are giving rise to the new form, are such heterozygous individuals, and that our new form is really a supposed extinct

form, whose existence is revealed through segregation of unit characters in the gametes of the heterozygous individuals. But this conception was unsatisfactory as a present working hypothesis, since it necessarily assumes a parent form, the existence of which can only be proved by paleontological records. So far as the writer has been able to ascertain, none of the extinct species of *Juglans* or *Carya* thus far described resemble the new form in number and shape of leaflets and proportionate size of lateral and terminal leaflets.⁴ Herbarium material of other related genera has also been examined to see whether a suggestion of the new form's leaf characters could be found, but without success. However, until some other hypothesis is proved to fit the case, this one should be reserved as having some value.

Another possibility is that the new form is a hybrid between *Juglans californica* and some other species, such as *J. regia* or *J. nigra*. This suggestion has not been used as a working hypothesis because the characters of hybrids between these two species and *J. californica* are already known. It is generally understood that plants of the F_1 generation of both these crosses reveal the partial dominance of *J. nigra* or *J. regia* as the case may be.⁵ They always have larger leaves than those of *J. californica*, whereas our new form is characterized by its small leaves. It is hardly conceivable, then, that the new form is the direct result of such a cross, nor even that the wild trees from which our new form springs could belong to the F_1 or a later generation from such a cross and thus produce the new form among the extreme variations that sometimes occur among the younger generations of hybrids between species. For our new form is the only extreme variation which has been reported among the many thousands of *J. californica* seedlings that are grown annually in California. Moreover, *J. regia* has been cultivated in southern California for less than fifty years, while only a few cultivated trees of *J. nigra* occur, and these also came with the introduction of commercial walnut growing. Other known species of *Juglans*

⁴ Dr. W. A. Berry, in a letter to the writer, expressed the following opinion: "I suspect if a paleobotanist had come across such a form, and I know of none such, he would have thought of the Anacardiaceae rather than *Juglans*."

⁵ Smith, R. E., "Walnut Culture in California," Univ. Calif. Agr. Exp. Sta. Bull. 231, pp. 157-170.

are also sufficiently distinct from *J. californica* so that hybrid offspring of the F_1 generation would hardly resemble our new form. Exceptions to this would be *J. rupestris* and *J. major*, the Texan and Arizonan species, which resemble *J. californica* rather closely, and on this account the possibility of our new form being an F_1 hybrid between either of these species and *J. californica* is even less likely. Hence, while it is very desirable that a systematic study of hybridization among all species of *Juglans* should be made, because of its botanical, genetic and horticultural interest, the writer does not consider it likely that the new form is a hybrid between *J. californica* and any other known member of the genus. By analogy we may eliminate all other members of the family Juglandaceae from similar participation in the origin of the new form.

Turning next to the three hypotheses which serve as a basis for the investigation now in progress, it would seem that sufficient work along any one of these lines might lead to a solution of the problem. They will be discussed in the order in which they were originally taken up by the writer. They are as follows:

(1) The new form may be a natural hybrid between *Juglans californica* Wats., and *Quercus agrifolia* Née, or some other oak.

(2) The new form may originate in certain teratological flowers that have been discovered on certain *Juglans californica* trees.

(3) The new form may be the result of mutations in certain male or female flowers (or both) of certain *Juglans californica* trees.

FIRST HYPOTHESIS

Let us consider first the possibility of origin through hybridization between walnut and oak. The original trees were first shown to the writer as "crosses between a walnut and an oak." They were briefly described by him under the title "The Walnut-Oak Hybrids," in Jepson's *The Silva of California* (pp. 50-54). In 1907, N. B. Pierce verbally expressed to the writer his opinion that the new form is a hybrid. Since the conception of hybrid origin was entertained by various persons, it seemed wise to investigate the possibility, not only of the occurrence of natural

hybrids, but also of producing such hybrids artificially. From the descriptions of the two families it is evident that they are rather closely similar. Following is a table of comparison⁶ of the reproductive organs in *Juglans* and *Quercus*:

<i>Juglans</i>	<i>Quercus</i>
<i>Plants</i> monoecious.	<i>Plants</i> monoecious.
<i>Staminate flowers</i> on lateral pendulous catkins on last season's wood; calyx irregularly 3 to 6-lobed; stamens numerous.	<i>Staminate flowers</i> on pendulous (except in one species) catkins from buds of the previous season; calyx parted into several lobes; stamens 4 to 12.
<i>Pistillate flowers</i> solitary or few in a short terminal spike; calyx 4-lobed, adherent to the 1-celled inferior ovary; styles 2.	<i>Pistillate flower</i> 1 in an involucre; involucre 1 or 2 in the upper axils of the season's shoot; calyx adherent to the 3-celled, 6-ovuled ovary; ovary with 3 to 5 styles or stigmas.
<i>Fruit</i> a 1-celled, incompletely partitioned nut, 1-seeded, the seed so lobed as to fit the irregularities of the cavity, exterior of nut covered with green and fleshy or at length dry and brown husk.	<i>Fruit</i> a 1-celled, 1-seeded nut, only 1 ovule maturing; seed with thick, fleshy cotyledons; the nut set in a sealy cup.

From the above table it is evident that in gross structure of flowers the two genera are closely similar. Of course, there is always the possibility or likelihood that some feature in the minute structure of the pistillate flowers or in the physiology of fertilization may absolutely prevent hybridization between any and all species of the two genera. The writer has not yet been able to engage in the cytological study necessary to confirm or deny this possibility.

The first effort made was to search for chance natural hybrids in the wild. In 1907 about four thousand nuts of *Juglans californica* were gathered in Brea Cañon. They were taken from trees standing close to coast live oaks. As they were planted late in the spring they were slow in germinating and cutworms destroyed many of them, but among two hundred that grew during the summer of 1908, no aberrant forms appeared. No further efforts in this direction were made, as the possibilities of success were considered too remote to warrant the expense of collecting and growing.

⁶ Based on descriptions of the genera in Jepson's *The Silva of California*.

In undertaking cross-pollination experiments it was reasoned that the failure of a large number of careful efforts to secure such a hybrid would discredit this hypothesis, while the production of one such hybrid artificially would tend to strengthen it. During the first year's work the only oak experimented with was the coast live oak, *Quercus agrifolia*, but in succeeding years one or more other species were included in the trials. The female flowers used were all on *J. californica* trees in 1908, but since that year an effort has been made (yet without success) to secure reciprocal crosses.

Experiments in 1908, 1909, and 1910

Two indigenous walnut trees were selected. They were located, one at the rear and the other at the front of a large city lot in the suburbs of Los Angeles. Through the courtesy of the residents, they were protected from interference during the critical stage of the work. They will be designated as Tree I and Tree II.

Manila paper bags were placed over the pistillate catkins almost as soon as they appeared and before pollen was being shed by the staminate catkins. Oak pollen was collected in homeo-

SUMMARY FOR 1908

Tree I					
Source of pollen	Number of pistillate catkins pollinated	Number of catkins on which nuts formed	Number of nuts produced	Nuts that germinated in 1909	Trees growing in 1913
<i>Q. agrifolia</i>	23	14	27	26	24
Freak S.	19	8	13	13	12
Checks	8	0	0	0	0
Tree II					
<i>Q. agrifolia</i>	17	16	37	33	32
Checks	5	1	2	2	2

pathic vials and applied with camel's-hair brushes. In 1908 no pollen was being shed on Tree I, even at the time of pollination. On Tree II pollen was being shed but care was used not to expose

the pistillate flowers when pollinating them. The only other source of pollen in 1908 was Disher's original sterile tree, which is referred to below as Freak S. In the following table "checks" are bags that were left in place at time of pollination and allowed to remain for several weeks, in order to learn whether nuts would develop. The trees referred to as growing in 1913 are located on the campus of the University of California.

In 1909, the walnuts were very late in blooming and conditions were so adverse that no nuts whatever were produced as a result of pollinating thirty-four different catkins (about sixty flowers).

In 1910 conditions were favorable and very promising. In May, 1910, there were 151 nuts developing as a result of pollinating 79 pistillate catkins with *Quercus agrifolia* pollen, and 29 nuts as a result of pollinating 16 pistillate catkins with *Q. engelmanni* pollen. But through a miscalculation on the part of an assistant regarding the proper time to secure the nuts before they dropped from the trees, the entire lot was lost. However, this assistant, S. E. Goodall, made similar experiments at his home near Chatsworth, using pollen from *Q. agrifolia* and *Q. lobata*. The oak-pollinated nuts were protected and saved, and as a result there are growing on the campus of the University of California eight young trees from *Q. agrifolia* pollinations and four from *Q. lobata* pollinations.

Experiments in 1911

The trees used are located on F. Goodall's ranch near Owensmouth, Los Angeles County. Three different trees were used. They will be designated by the letters A, B, and C. Coffee bags, having the outer layer of oiled paper, were used to cover pistillate flowers. An effort was also made to secure reciprocal hybrids. One tree of *Quercus lobata* was found which was shedding but little pollen and which was somewhat removed from others of its kind. Bags were placed over seven shoots of the season's growth, thus covering forty to fifty pistillate flowers, after first having pollinated them with *J. californica* pollen. Three weeks later the bags were removed and some flowers seemed likely to develop further, although some were moldy. On July 21 all had dropped.

SUMMARY FOR 1911

Tree A			
Source of pollen	Number of pistillate catkins pollinated	Number of nuts produced	Nuts germinated June 1, 1912
<i>Q. agrifolia</i>	20	9	0
<i>Q. lobata</i>	20	9	0
<i>Q. dumosa</i>	12	4	2
Checks	14	4	0
Tree B			
<i>Q. agrifolia</i>	24	18	15
Checks	13	10	7
Trees B and C ⁷			
<i>Q. lobata</i>	44	29	22

Results of Hybridization Experiments

From the 1908 pollinations with *Quercus agrifolia* upon *Juglans californica* there are now 48 four-year-old seedlings. Clons from most of these have been grafted this spring upon large English walnuts in order to hasten fruiting. It is planned to protect these grafts at the blooming period so as to secure self-fertilization and thus make possible the "breaking-up" which may occur in the F_2 generation if they are really hybrids.

From the 1910 experiments (made by Goodall) there are eight seedlings from the *Q. agrifolia* pollinations and four from *Q. lobata* pollinations. Grafts may be made from these next year.

From the 1911 experiments there are 14 seedlings from the *Q. agrifolia* pollinations, 20 from the *Q. lobata*, and 2 from the *Q. dumosa* crosses.

The seedlings obtained from all oak pollinations resemble *J. californica* in leaf characters and habit of growth. This does not necessarily mean that these seedlings are not true hybrids. It has been demonstrated that certain species-hybrids are goneoclinic to the female parent in the F_1 generation.⁸ It is in the hope of showing that these seedlings are hybrids by the appearance of oak characters in plants of the F_2 generation that the seedlings are being propagated to secure early fruiting.⁹

⁷ While in storage the *Q. lobata* nuts from Trees B and C became mixed, but 24 of the 29 came from Tree C.

⁸ Keeble, F., *The Gardener's Chronicle*, vol. 52, no. 1355, p. 457.

⁹ Since writing the above it has been found that most of the seedlings from the 1908 crosses bloomed this spring (1913). Hence it was possible to secure self-pollination on the seedlings themselves at an earlier date than was anticipated.

In 1908 the cheek results were much more satisfactory than in 1911. Various factors may have influenced these results, but the writer is inclined to think that the comparative isolation of the Los Angeles trees made them more satisfactory subjects for experimentation. From the 1911 results we find nearly as high a percentage of nuts in cheek bags as in pollinated bags from Tree A and a higher percentage from Tree B. The production of nuts in the cheek bags raises the question of the possibility of apogamic development of seed in *Juglans californica*, and throws a shade of doubt upon the supposed hybrid seedlings that have been obtained from oak pollinations. In this connection more experimental work should be done in preventing natural pollination on a large scale.

For the present the writer is inclined to think that one of the other two hypotheses is much more likely to reveal the origin of our new form. Dr. Cannon, of the Desert Laboratory, Carnegie Institution of Washington, who is an authority on plant hairs, informs me that he finds no evidence of oak characters in the hairs of Disher's original trees.

SECOND HYPOTHESIS

Let us consider next the possibility that the new form may originate in certain teratological flowers on certain *Juglans californica* trees. It is necessary to give here a brief account of the discovery of these abnormal flowers and to state the reasons for considering them as a possible source of the new form. In order to emphasize the teratological features about to be described, it may be well to consider first the characteristics of normal fruits and flowers. Normal flowers are shown in plate 6, figure 10. Normal leaves and fruits are shown in plate 7, figure 11.

The normal blooming period of most wild black walnut trees in southern California is in April. As the staminate catkins are produced upon the wood of the previous season's growth, their gradual increase in size may be observed at any time during late winter or spring. During March they lengthen noticeably. About April first the pistillate catkins appear, terminating the first new growth of the season. They are one or two inches long and bear one to five flowers, so that, when the fruits mature,

they often hang in clusters (cf. plate 7, figure 11). Normal pistillate flowers are bisymmetrical. Normal staminate catkins are two to four inches long, pendulous, and bear an indefinite number of flowers (cf. plate 6, figure 10).

Many trees throw out lateral branchlets from the first growth of the season. It is usually during this second period of growth, in May or early June, that teratological flowers and leaves appear on certain trees. (It should be noted here that abnormal female flowers have been observed by the writer during the *normal* blooming period of two or three trees. A note on such specimens with illustrations appears in Jepson's *The Silva of California*, pp. 55, 56. So far as is known these flowers never produce fruits containing viable seeds.) The late or second-growth abnormal flowers are usually produced upon catkins that resemble normal *staminate* catkins in number and arrangement of flowers. But the flowers are either *pistillate* or *bisexual*, often both occurring on the same catkin. Only a few staminate flowers have been observed among these late blooms, and these were on catkins which were entirely staminate. One tree was observed on May 29, 1909, on which nearly all the late or second-growth catkins were staminate, but the flowers were dropping instead of developing to maturity. Most late-appearing catkins occur on the second-growth, lateral branchlets, one catkin in the axil of a leaf, but they sometimes develop alongside the normal, terminal, pistillate catkin, as shown in plate 8, figure 12. In Brea Cañon (Puente Hills) a hundred or more wild trees were examined during the season of 1909, and, while abnormal flowers and fruits were not of general occurrence, they were found to be very frequent.

The most striking characteristic of the pistillate and bisexual flowers is their form. They are asymmetrical, being flattened more or less on the side adjacent to the axis of the catkin. Along with this flattening there is often a depression in the surface of the ovary, usually extending from the styles to the base of the ovary or only part way, and of varying width (cf. plate 8, figure 12).

When teratological flowers develop into fruits, the asymmetrical form and principal surface markings are retained. Over fifty

abnormal fruits, with their husks dry and brown but in most cases uninjured, were picked up by the writer beneath one of the several trees upon which such fruits have been known to mature. But most of these trees produced only a few such fruits. Mature abnormal fruits are easily assorted into two or three lots according to their external markings. Very few differ notably from the two classes illustrated in plate 9, figure 13. However, in each class there are greater extremes of variation than are here shown. The series *a, b, c* has been designated "Type X" and the other series, "Type Y." In *a, b, c, d, e*, the fruits appear as when picked up; *a, b, c*, are seen in longitudinal plane, while *d* and *e* are seen from above; in *a', b', c', d', e'*, the dry husk has been entirely removed from the woody shell of the nut. These nuts are from fruits which resemble *a, b, c, d*, and *e* so closely as to be practically identical. While *a', c', e'* are seen from above, *b'* and *d'* are shown in longitudinal plane. The nuts *a'', b'', c'', d'', e''* are practically identical with *a', b', c', d'* and *e'*. They were sectioned transversely and placed in relatively the same positions as *a', b', c', d'* and *e'* respectively, the basal portion of the nut being placed in the upper of the two rows in each case. To me, the most striking abnormality shown in this picture is the unequal reduction of the cotyledons in all except *e''*.

Two culture tests of abnormal nuts selected to these types have been made in 1910 and 1911. There is considerable variation among the seedlings grown from each type, but no general differentiating character could be found among all the seedlings from the two types of nuts. These tests were subsidiary to the general trials of abnormal nuts conducted during the same years in order to ascertain whether the new form originates from such nuts. Among sixty-eight seedlings growing not one has shown the slightest indication of the leaf characters of the new form. In 1910 trials one seedling appeared that had three-parted scale-like leaves, but it died before it was three inches high. The leaves did not resemble those of the new form.

On otherwise normal *Juglans californica* trees, abnormal leaves have been observed in two situations—near the base of second-growth lateral branchlets and, occasionally, associated with the abnormal catkins already described. Abnormal leaves, from

second-growth lateral branchlets on trees bearing abnormal flowers, and leaves associated with abnormal catkins are shown in plate 9, figure 14. Some of these leaves resemble somewhat the typical leaves of the new form and, at the same time the leaves shown here were collected, one second-growth lateral branchlet was found that bore a leaf very similar to the typical leaf of the new form (cf. plate 10, figure 15, *a*).

We may now summarize the phenomena observed in connection with the occurrence of teratology in *Juglans californica* and add some considerations with respect to the possible origin of the new variety. Abnormal flowers, fruits and leaves are of frequent occurrence on indigenous trees. They usually occur later in the growing season than the normal blooming period, on secondary, lateral branchlets, or in the case of abnormal catkins, sometimes as secondary catkins terminating the first growth of the season. The fruits produced by abnormal flowers retain the characters of asymmetry and irregularity of surface. Their average size is only about half that of normal fruits.

Only a small percentage of the abnormal fruits collected by the writer contained viable seeds. Among all the seedlings which have been grown from abnormal nuts, not one shows a trace of the leaf characters of the new form. On the other hand, in connection with the fourth recurrence of the new form at Garden Grove in the spring of 1911, the writer was able to ascertain definitely that some of the seedlings of the new form grew from nuts of normal size and shape. These facts indicate that the new form does not originate from teratological fruits. However, we must concede the possibility of the original trees having so originated. More of these abnormal nuts should be collected and the seeds tested.

The reasons for thinking that the new variety may have originated from teratological fruits may be concisely stated as follows:

1. There is more or less similarity between the abnormal leaves, found on secondary branchlets or associated with abnormal catkins, and the leaves of the new form.

2. Abnormal flowers and fruits are frequently found during the *normal* blooming period on some of the original trees of the

new form and in considerable variety, especially the flowers, many of which do not mature into fruits. Bisexual flowers are rather frequent and, whether bisexual or not, the flowers often have peculiar external markings on the ovary resembling those already noted in the teratological flowers above described.

3. *Late* flowers, i.e., abnormal catkins on second-growth wood, have been observed on four of the seven original trees of the new form examined by the writer. The other three trees seldom bear any flowers. Specimens of these abnormal catkins collected from the Leffingwell original tree in 1909 are shown in plate 11, figure 17. From the size of the normal young fruit shown at *a*, the difference in time between the normal blooming period and the appearance of these abnormal catkins may be inferred. The flowers on these abnormal catkins are very small and abortive and the leaves, shown at *a*, *b*, *c*, and *d*, resemble the leaves shown at *d*, *e*, and *f* in plate 9, figure 14, which were associated with abnormal catkins on indigenous *Juglans californica* trees.

4. There is no apparent obstacle to the natural pollination of the late-appearing teratological flowers, as some of them are bisexual and free pollen has been observed in these bisexual flowers. Also a few late staminate catkins have been found. Moreover, there is wide variation in the normal blooming period among individual, indigenous trees. The young fruits of different trees have been observed to vary in size from nearly full-grown down to five-eighths of an inch in diameter. It is possible, then, that the normal pollen produced on late trees might fertilize abnormal flowers on early blooming individuals. However, in the test for apogamic development of normal fruits it would be interesting to test these teratological flowers also.

The reasons for not thinking the new variety originated in late teratological flowers are as follows:

1. Sixty-eight seedlings have been grown from teratological nuts and none have resembled the new form.
2. The new form is known to have been produced by nuts of normal size and shape.

From the data at hand, the writer is inclined to consider the second hypothesis as approaching more nearly to the truth than the first, but thus far direct evidence fails to support it.

THIRD HYPOTHESIS

The possibility that the original trees of the new form are mutants has been recognized from the first. If it can be shown that the recurrence of this form among seedlings of *Juglans californica* trees is due to repeated mutations in certain individuals, this would be good evidence that the original trees were similarly produced. The repetition of mutations is not contrary to experience but rather is characteristic of species known to be in a mutating period. It is generally admitted that plants exhibiting the evidence of being in a mutating period are rare. "Hugo de Vries admet l'hypothèse d'une mutabilité périodique et rare" (Blaringhem). Among the plants reported as being in such an abnormal condition, the number of trees is very small. Hence the phenomena connected with the occurrence and recurrence of this new form of walnut gather increased interest as soon as the hypothesis of origin by mutation is considered.

The chief obstacle to adequate investigation based on the hypothesis has been the difficulty in locating a single tree of *Juglans californica* from whose seeds the new form is known to grow. With such a tree located under conditions favorable to experimental work, a careful study of its vegetative and reproductive parts can be made and pollination may be controlled. After several years of searching such a tree has now been located. It is a certain tree in the Garden Grove row of black walnuts¹⁰ from which nuts have been gathered by nurserymen, who have found seedlings of the *quercina* type during a period of several consecutive years.

In 1912 I had the nuts from twenty-one of the trees in this row gathered separately, labeled with numbers corresponding to numbers attached to the trees, and shipped to Berkeley. These were soaked, planted in sand in flats, properly labeled and placed under the benches of a glass house, in February, 1913. Two months later, six *quercina* seedlings were found in the flats from tree No. 16. There were 275 seedlings in all. Thus slightly over two per cent of the seedlings secured from the 1912 crop of this tree appear to be mutants. Plate 11, figure 18, shows one of these seedlings and one normal seedling from tree No. 16. Plate

¹⁰ Cf. Recurrence of the New Form, p. 5.

12, figure 19, is from a photograph of tree No. 16 taken in April, 1913. About four hundred twigs on this tree likely to bear nuts have been covered so as to insure self-pollination. In this way it is hoped to locate the twig or twigs producing mutants.

The data at hand indicate that the new form is not a typical mutation from the seed in the usual meaning of that expression. All but one of the tests of the seeds from the original trees have revealed a partial reversion to the species type in the second generation (counting Disher's original trees as the first generation). Now, one of the generally accepted distinguishing characteristics of a mutant is that it breeds true from the first, but here is a remarkably distinct form which does not breed true. Of course, this should not exclude the possibility of origin by mutation of an "eversporting variety." It is quite possible that *quercina* is an eversporting variety and, if so, it may still have originated without an antecedent hybridization, but would then *never* breed true.¹¹ However, it does not appear to the writer that the term "eversporting variety" as used by De Vries is applicable to this form. De Vries classes as eversporting varieties such inconstant forms as striped flowers, five-leaved clovers, and polycephalic poppies. But in this new walnut, we have a form which is distinct in most of its characters from the parent form and which breeds true in a portion of its offspring, the remaining portion showing complete resemblance to the parent form.

It has been suggested by Professor H. B. Torrey that mutations may occur in the gametes of one sex while the gametes of the other sex are normal and, hence, that the new form appears among first generation seedlings, but fails to breed true in the second generation.

Some doubts as to Professor Torrey's suggestion arise when we consider the results of a pollination experiment which the writer made in 1908. Pollen from Disher's original non-fruited tree was placed upon pistillate flowers of Tree I, referred to in the discussion of the first hypothesis. All of the eleven seedlings growing from this cross resemble *Juglans californica*. On the basis of Professor Torrey's suggestion, this would be explained

¹¹ Dr. George H. Shull kindly suggested the explanation on the basis that *quercina* is an eversporting variety.

by assuming that the pollen grains engaged in fertilization bore *J. californica* characters as a result of segregation in the reduction divisions. But it is as reasonable to explain these results by assuming that the ovule-borne characters of *J. californica* are dominant or prepotent over the pollen-borne characters of the new form, supposing that fertilization actually took place. It is obvious that more extensive experiments should be made in an effort to secure reciprocal crosses between the species and the variety.

Webber has expressed the following opinion: "Of the various causes of origin it seems to me most reasonable to assume that it is a mutant and the type of mutation to nanate form is similar to *Oenothera nanella* and the Cupid Sweet Pea. One finds parallel cases of partial reversion to the parent type among De Vriesian mutations."

The reasons for thinking the new form may have originated through mutations in otherwise normal flowers of *J. californica* may be stated briefly as follows:

1. In the 1911 recurrence of the new form in Garden Grove, it was found by actual examination of the seed bed that all of the aberrant seedlings examined grew from nuts of normal size and shape.

2. The crop of 1912 from a certain tree of *Juglans californica* (No. 16 in the Garden Grove row above mentioned) has produced several seedlings of the new form. The nuts from which they grew are of normal size and shape. The possibility of hybridization with any species of oak or other species of walnut is very remote.

3. The large tree standing close to No. 16 is known to have been the source of many of the nuts planted in Tyler's nursery in the years when *quercina* seedlings appeared in his seedbeds. On this account it was suspected of being the source of the new form. It appears, however, that No. 16 has been the source of some *quercina* seedlings and that the particular seeds that gave rise to it were produced on the branches nearest the tree originally suspected.

Transmitted May 9, 1913.

PLATE I

Juglans californica var. *quercina* Babcok

Fig 1.—Disher's original trees in Garden Grove, Cal. The one at the left bears regularly, the other produces only staminate or very rarely abortive pistillate flowers.



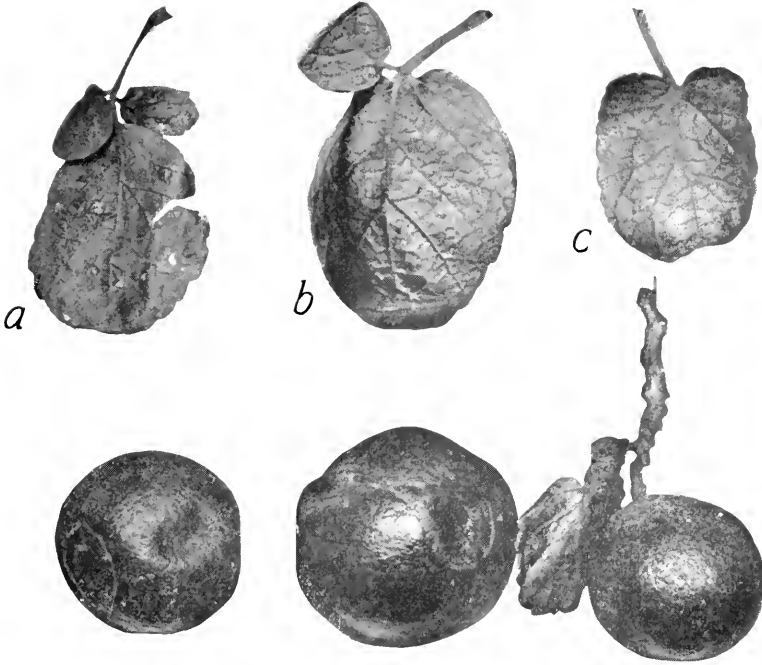
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PLATE 2

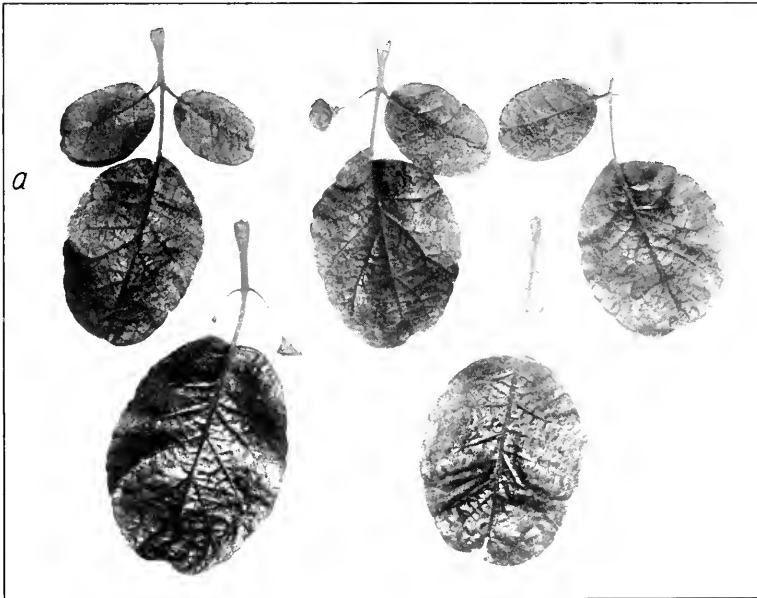
Juglans californica var. *quercina* Babcock

Fig. 2.—Leaves and nuts from Disher's original fertile tree. $\times 1$.

Fig. 3.—Normal leaf (*a*) from the Leffingwell original tree and four leaves from the same tree bearing ascidia. $\times 1\frac{1}{2}$.



2



3

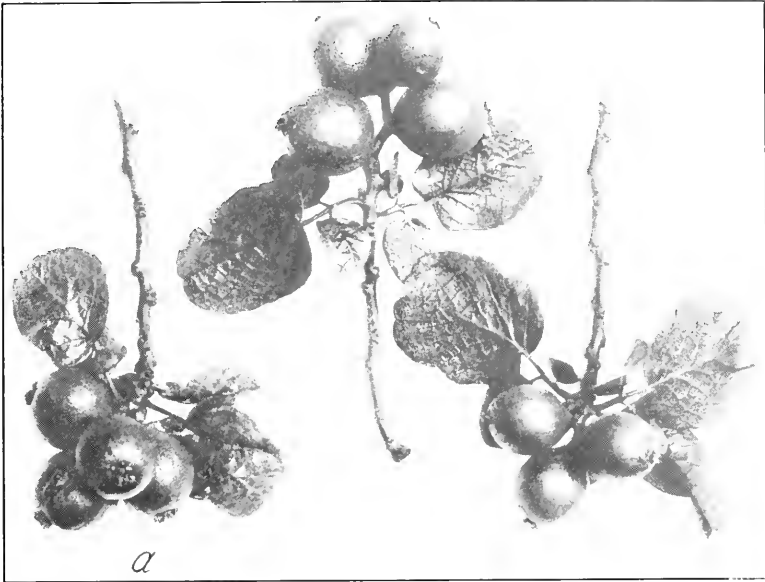
PLATE 3

Juglans californica var. *quercina* Babcock

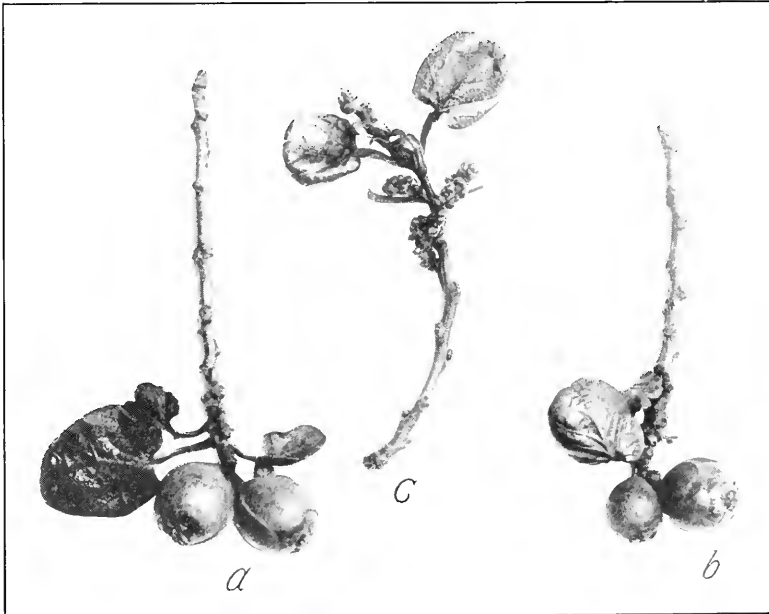
Fig. 4.—Twigs with partially developed fruits from Disher's original fertile tree. $\times \frac{3}{4}$.

Juglans californica var. *quercina* Babcock

Fig. 5.—Twigs with fruits from Disher's original fertile tree and a twig bearing late flowers from the same tree. $\times \frac{3}{4}$.



4



5

PLATE 4

Juglans californica Wats.

Fig. 6.—*a, b, c, d, e*, nuts sectioned transversely; *f*, exterior of one nut. $\times 1$.

Juglans californica var. *quercina* Babcock

Fig. 6.—*g, h, i, j, k*, nuts sectioned transversely; *l*, exterior of one nut. $\times 1$.



a



b



c



d



e



f



g



h



i



j



k



l

6

PLATE 5

Juglans californica var. *quercina* Babcock

Fig. 7.—First recurrence of the new form. A two-year-old seedling at Garden Grove in 1907.

Fig. 8.—Leaf from another seedling of the same lot as the one shown in fig. 7. $\times 1$.

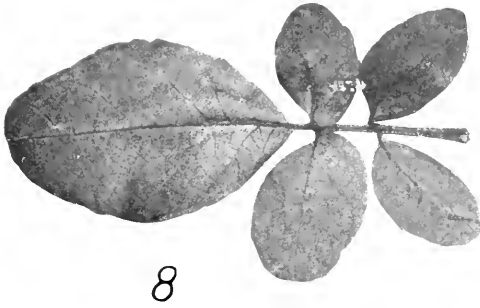


PLATE 6

Juglans californica var. *quercina* Babcock

Fig. 9.—Flowers from Disher's original tree in 1913; (*a*) male, (*b*) female. $\times 1$.

Juglans californica Wats.

Fig. 10.—Normal flowers; (*a*) male, (*b*) female. $\times 1$.

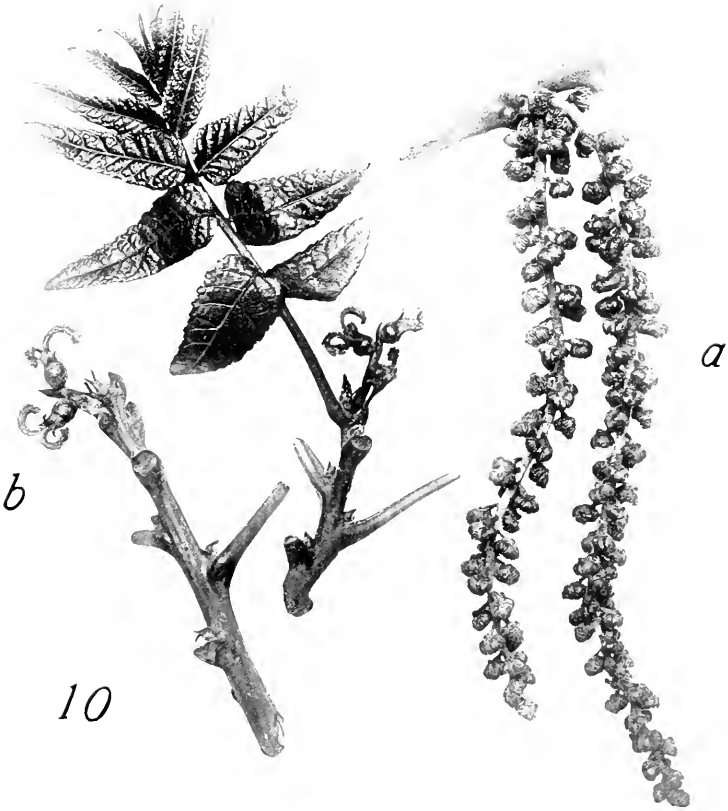
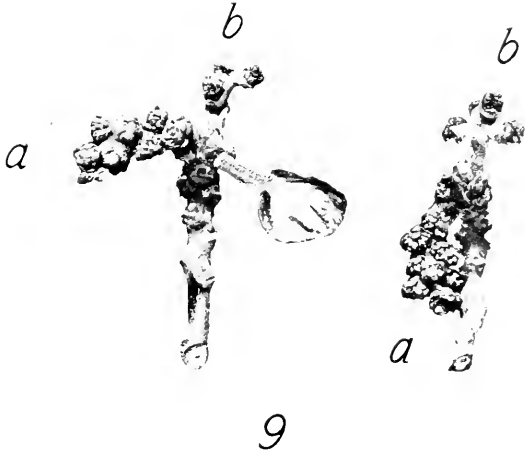


PLATE 7

Juglans californica Wats.

Fig. 11.—Normal leaves and fruits. $\times 1/2$.



PLATE 8

Juglans californica Wats.

Fig. 12.—*a*. Normal pistillate catkin with a developing nut; *b*. Abnormal catkin with pistillate flowers; *c*. Shows asymmetry of the abnormal flowers; *d*. Shows depression in surface of ovary; *e*. A bisexual flower. $\times 1$.

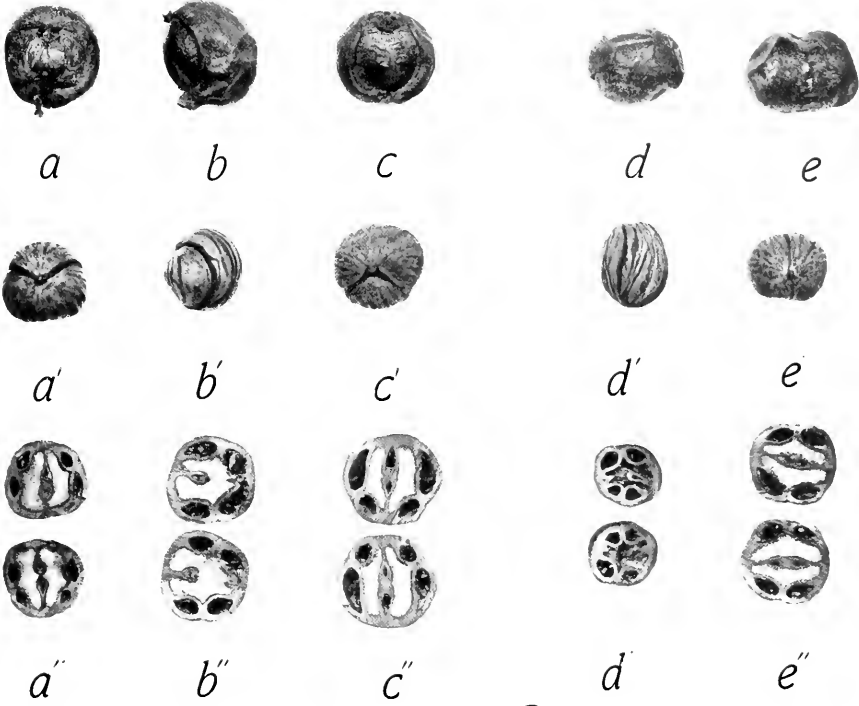


PLATE 9

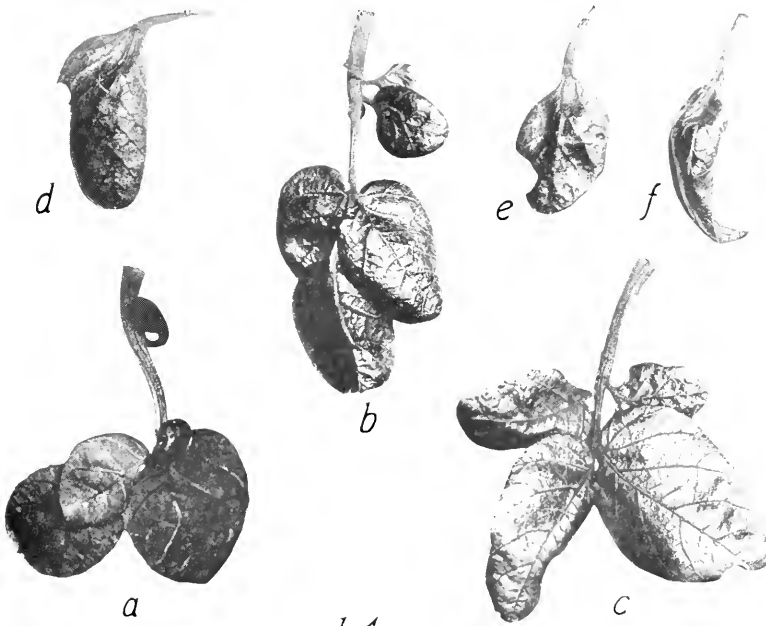
Juglans californica Wats.

Fig. 13.—Abnormal fruits—*a, b, c*, type *x*; *d, e*, type *y*.
d', b', e', d', e'. Abnormal nuts from similar fruits.
a'', b'', e'', d'', e''. Similar nuts sectioned transversely and placed in relatively the same position, the basal portions being uppermost in the figure. All $\times 1\frac{1}{2}$.

Fig. 14.—Abnormal leaves—*a, b, c*, from second-growth lateral branchlets; *d, e, f*, associated with abnormal catkins; all collected from wild trees in Brea Cañon in 1909. $\times \frac{3}{4}$.



13

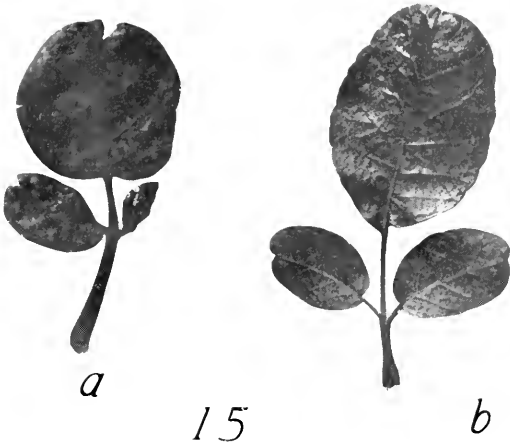


14

PLATE 10

Fig. 15.—*a*, Abnormal leaf on a second-growth lateral branchlet on *Juglans californica*, Brea Cañon, 1909; *b*, a typical leaf from the Leffingwell *quercina* tree. $\times \frac{2}{3}$.

Fig. 16.—One of the three original *quercina* trees at N. B. Pierce's gardens in Santa Ana, 1913.



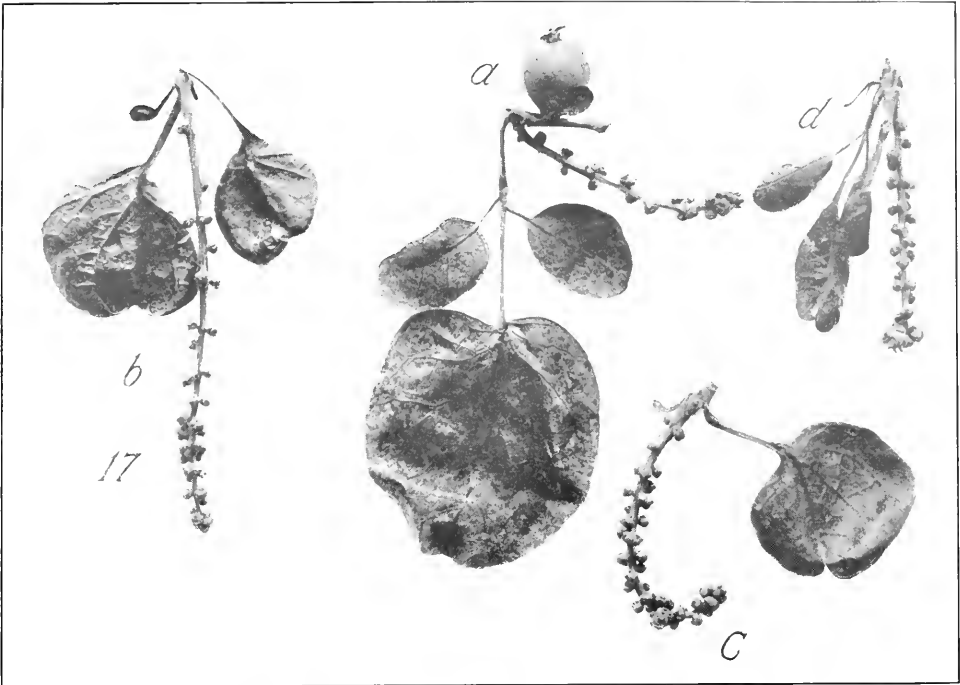
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PLATE 11

Juglans californica var. *quercina* Babcock

Fig. 17.—Abnormal late catkins from the Leffingwell tree; *a*, normal catkin bearing one young fruit and with an abnormal catkin growing from its base; *b*, *c*, *d*, abnormal catkins and leaves. $\times \frac{2}{3}$.

Fig. 18.—Two seedlings from *Juglans californica* tree No. 16 (Garden Grove), March, 1913; *a*, typical of the species; *b*, *quercina* form. $\times \frac{1}{2}$.

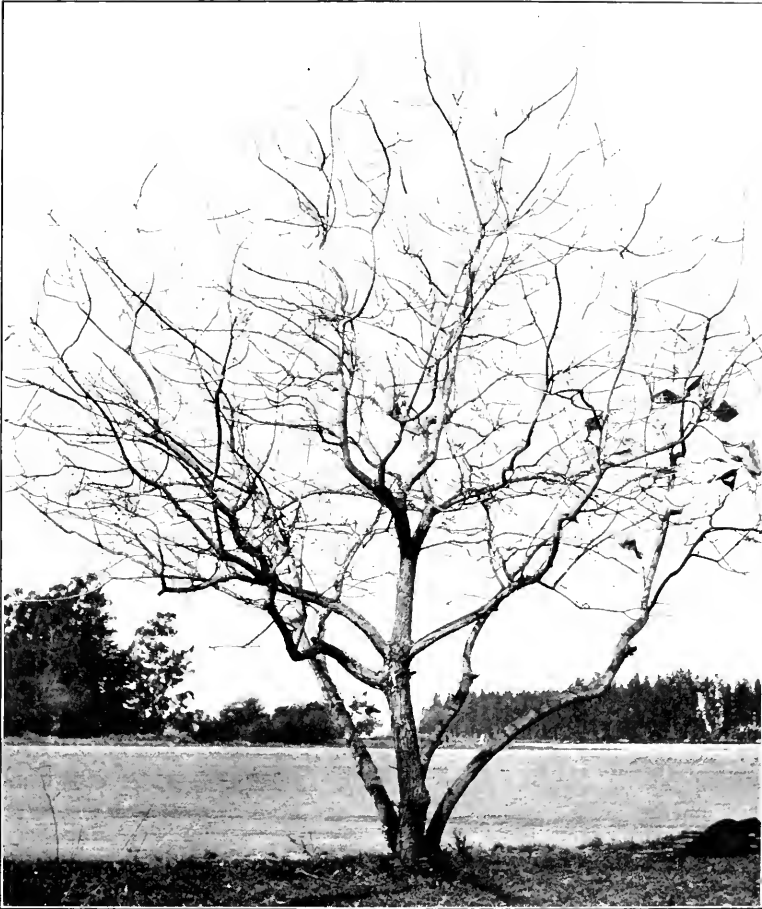


a 18 *b*

PLATE 12

Juglans californica Wats.

Fig. 19.—Tree No. 16, Garden Grove, April, 1913.



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STUDIES IN JUGLANS II

FURTHER OBSERVATIONS ON A NEW VARIETY OF
JUGLANS CALIFORNICA WATSON AND ON
CERTAIN SUPPOSED WALNUT-OAK
HYBRIDS

BY

ERNEST B. BABCOCK

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STUDIES IN JUGLANS II

FURTHER OBSERVATIONS ON A NEW VARIETY OF *JUGLANS CALIFORNICA* WATSON AND ON CERTAIN SUPPOSED WALNUT-OAK HYBRIDS

BY

ERNEST B. BABCOCK

In the preceding paper on this subject¹ appeared the history and description of a new form of California Black Walnut, which was named *Juglans californica* var. *quercina*, together with a discussion of several hypotheses regarding its nature and origin. The following hypotheses were deemed worthy of serious consideration and feasible as bases for further study:

(1) Origin through hybridization with the Coast Live Oak, *Quercus agrifolia* Née.

(2) Origin from teratological flowers and fruits of *J. californica*.

(3) Origin by mutations in apparently normal flowers and fruits of *J. californica*.

In order to test the first hypothesis, that of origin through hybridization between walnut and oak, attempts to hybridize the two species were made in the years 1908 to 1911, inclusive. As a result of the pollination work in 1908, fifty-six seedlings were secured from nuts that developed from pistillate walnut flowers which had been pollinated with oak pollen under bag. Except for considerable variation in size, these trees have not exhibited more differences than would be found among ordinary trees of

J. californica growing in the wild. However, since in certain known interspecific hybrids the F_1 plants all resemble the female parent, it was deemed advisable to grow at least the second generation from all these trees. In 1913 thirty of them bore nuts, which were collected and stratified. Also in the spring of 1913 large paper bags were placed on most of these trees enclosing flowers of both sexes in order to insure self-pollination of a few flowers on each tree. These bags were shaken vigorously several times during anthesis and upon examination of several flower clusters the stigmas were found to be well covered with pollen. Very few nuts developed under bag, but this may not have been due to self-sterility on the part of the individual trees. A period of very warm and humid weather caused most of our walnut flowers that were developing under bag, both in Southern California and at Berkeley, to drop. From the self-pollinated nuts secured on these supposed hybrids 75 seedlings from 24 different trees are growing. These, together with 2001 seedlings from naturally pollinated nuts representing 30 different trees, give a total of 2076 second generation seedlings that have been examined. Among them all not a single individual has been found that resembles the new variety. Neither is there any indication of oak characters in these F_2 seedlings. Therefore as yet we have no evidence that the F_1 trees are true hybrids. A sufficient number of F_2 plants will be retained to grow the F_3 generation from each F_1 tree.

Now the question arises as to the nature of the supposed F_1 hybrids. I consider the conditions under which the work of pollination was performed in 1908 to have been practically ideal. The pistillate flowers were bagged long before any walnut trees were shedding pollen. On one of the two trees I am certain that no pollen was being shed even at the time I pollinated. This tree was fairly well isolated from other walnuts, some of which were shedding pollen at that time. Moreover, no nuts developed in the check bags on this tree. Now if natural pollination was prevented both before and after the application of oak pollen, why are not these trees hybrids? Are they the result of abnormal embryogeny due perhaps to stimulation by the oak pollen but without the occurrence of fertilization? It should

be remembered that they are the progeny of only two different trees and, if they are the result of asexual reproduction, it would be reasonable to expect among the offspring of either parent marked uniformity in size, leaf characters, time of putting forth and shedding leaves, time of flowering and flower characters. But the variations in the above mentioned characteristics are so great as to suggest heterogeneous parentage within the species rather than asexual reproduction from one or two parent trees. Plate 13, figure 1, and plate 14, figure 2, show a typical leaf and a cluster of partly developed fruits from each of six of these F_1 seedlings.

With these facts in mind, let us consider briefly the various processes of abnormal embryogeny that may have given rise to these variable seedlings. (1) The new sporophyte may have developed from the megaspore mother-cell, in which case its cells would possess the diploid number of chromosomes characteristic of *Juglans californica*, provided that the parent tree was typical of the species. (2) It may have arisen from the megaspore, from the egg nucleus, or from one of the other embryo sac nuclei, without fertilization, in which case its cells would contain the haploid number of chromosomes. (3) It may have arisen adventitiously from sporophytic tissue, in which case its cells would contain the diploid number of chromosomes. It is obvious that in either the first or third instances we should expect much uniformity among the progeny and close resemblance to the parent trees. Hence, in view of the wide variation mentioned above, it is reasonable to assume the occurrence of one or more of the three phases of parthenogenesis included in the second of the three cases above defined.

Finally, this hypothesis of origin by hybridization with oak is practically annihilated by the discovery previously reported that in a row of twenty-one California Black Walnuts, growing in Garden Grove, Orange County, California, a single tree has been found which produces the new variety year after year. I know of no oaks in this region, but even if oaks were abundant and close at hand, the fact that *quercina* seedlings come from only one tree would certainly indicate some other cause than oak pollination.

As for the second hypothesis, that the new variety originated from teratological flowers of *J. californica*, this seemed very unlikely after testing sixty-eight abnormal nuts from several different trees and failing to secure it, and especially after discovering that the new form does originate from nuts of normal appearance. At the same time, the occurrence of teratological leaves, flowers, and fruits in this species would certainly indicate an unstable condition, which finds its most frequent expression in these abnormal features of the somatoplasm and which may occasionally result in such segregations in certain cell divisions preceding or accompanying gametogenesis as would result in what we call mutation. As a matter of fact the tree of *J. californica*, mentioned above, which has been under observation about two years (tree no. 16 mentioned at the close of the preceding paper), produced in 1913 a few clusters of these abnormal late nuts. These were found while gathering the normal nuts from this tree. Each cluster (produced on a single catkin) was gathered separately and given a number. A tree label bearing the same number was attached to the twig that bore that particular cluster. From each of three of these clusters of teratological fruits two or more *quercina* seedlings have appeared. Plate 15, figure 3, shows the seedlings grown from abnormal cluster number B4 which was borne on a late-appearing catkin which sprang from the base of a normal pistillate catkin on twig number 151. In this case there are four typical *californica* plants (*a*, *b*, *c*, *d*), four *quercina* plants (*e*, *f*, *g*, *h*), and two seeds that did not germinate. It may be questioned whether seedling *h* could be properly classified at such an early stage. Suffice it to say that, even at the time when the stem is just pushing through the soil, the appearance of *quercina* is quite distinct from that of *californica*. Seen from above the former has the shape of a rosette while the latter appears as a cone. This is due to the decided difference in the apices of the leaves (cf. *b* and *e*). Seedling *h* presents a fine exhibition of geotropism due to the position in which the seed happened to be planted. The extreme abnormality of some of the nuts of this cluster is strikingly shown in *a*, *c*, *e* and *f*. In each of these seeds one cotyledon was confined within a sector equal to about one-third the volume of the nut. It is

obvious that there is no relation between degree of abnormality of the nut and the appearance of the *quercina* form. However, the fact that *quercina* seedlings have been secured from teratological fruits might seem to indicate that in this fact of teratology one finds the basis for a complete solution of the problem in hand. But these are the first *quercina* seedlings I have raised from abnormal nuts, and of the twenty-eight seedlings secured only eleven are *quercina* in character. On the other hand, all the young *quercina* seedlings examined in the past have sprung from apparently normal nuts. This shows that something more than the mere fact of teratology must be found to explain the origin of the new variety.

Regarding the third hypothesis the evidence now at hand is definite and sufficient. Over three hundred clusters of normal nuts were gathered separately from tree No. 16 in 1913, following the method described above. The number of nuts per cluster varied from one to five. The nuts of each cluster were stratified in a pot bearing the same label as the twig from which the cluster came. Later the pots were transferred to a cool greenhouse where they were kept until the plants were several inches high. One *quercina* seedling was found in each of 42 pots. Plate 16, figure 4, shows the seedlings from the two nuts in cluster 35; plate 17, figure 5, shows the seedlings from cluster 97; and plate 18, figure 6, the seedlings from cluster 196. Besides the 600 seedlings grown from marked clusters of nuts, about 1000 additional seedlings were raised from this tree. Of the total number of seedlings grown approximately 5 per cent were *quercina*.

The fact that among the normal fruits of this particular tree only one nut in a cluster produces the *quercina* form, at once suggests a possible relation between location in the cluster and production of *quercina* seedlings. Observations and experiments are being conducted in order to determine whether there is any definite position in the cluster or other morphological feature that is associated with origin of the new form.

The nuts gathered from this tree in 1913 may not have been self-pollinated. About four hundred branchlets including staminate and pistillate flowers were bagged in early spring, but the warm moist weather mentioned above caused all the pistillate

flowers to drop. Thus my only recourse was to label and bag clusters of developing nuts. About 350 clusters were so treated. It is very likely that many of the flowers were cross-fertilized with pollen from neighboring trees. However, if the pollen of either no. 16 or its near-by neighbors had been the source of the new form, *quercina* seedlings should have been obtained among the progeny of other trees besides no. 16 in the original seed test made in 1912. Since only the one tree produces the new form I have disregarded pollination for the present, studying only the pistillate flowers and testing the seeds.

Cytological investigation will possibly reveal the true nature of the new form and perhaps explain its origin. Meanwhile the speculations concerning the nature of the supposed walnut-oak hybrids are suggestive in this connection. Through failure of pollination or fertilization parthenogenesis may take place. That polyembryony occurs both in the new form and the old is proved by the discovery of the specimens shown in plate 19, figures 7 and 8. In each case the two embryos were complete, each caulicle being attached to its own pair of cotyledons. Again, it is possible that at some stage in flower development abnormal mitosis occurs. Should this happen in a very early stage in the development of the flower, sufficient abnormal somatic tissue would be produced to make cytological investigation comparatively easy. It is hoped that by determining the morphological location of the nuts that produce *quercina* seedlings the cytological study of very young flower clusters will be somewhat simplified.

The present tendency² to refer to hybridization as the basis of all variation calls for a reference to my previous paper¹ where I discussed the hypothesis of origin through hybridization either with oak or with any form of *Juglans* and allied genera, and showed that such a hypothesis is untenable. The results reported in this paper bear out that conclusion. Further, as opposed to the proposition of assuming hybridization as necessary for the occurrence of mutation, we have the recent conclusion of Gates³ "that mutation and hybridization are separate phenomena, and that the cause of some at least of the mutations in *E. nothera* is independent of the combination of hybrid characters."

The origin of *quercina* is similar to those transmutations in *Lycopersicum*,⁴ *Gossypium*,⁵ *Nicotiana*,⁶ and *Oenothera*,³ which have been described as aggregate mutations as distinguished from loss or addition of single characters as, for example, in *Helianthus*⁷ and *Drosophila*.⁸ In regard to the tobacco mutation above referred to, the authors⁶ assume "that mutation must have taken place after fertilization, i. e., after the union of the male and female reproductive cells." Castle⁹ suggests it is equally probable that the mutation occurred in an egg cell which then developed without fertilization since "parthenogenesis is known to occur in tobacco and mutation in a growing or immature germ cell seems inherently more probable than in a fully formed and fertilized one." This discussion is pertinent to both phases of the problem herein set forth, viz., first, an explanation of the variation found in the F₁ oak-pollinated walnut seedlings; second, the cytological time of the mutations that produce *quercina* seedlings.

Concerning the first question, parthenogenesis or, more specifically, apogamy, assuming the occurrence of reduction in chromosome number, might explain the variation in the F₁ oak-pollinated seedlings whereas such an extent of variation is too great to permit the assumption that embryos developed from the spore mother cell or other sporophytic tissue without also assuming irregularities in chromosome behavior. Reduction is not assumed¹⁰ in the classical cases of parthenogenesis in angiosperms (*Antennaria*, *Taraxacum*, *Hieracium* and *Alchemilla*), but in *Thalictrum purpurascens* Overton¹¹ finds "the development and germination of the megaspore is that usually found among angiosperms." He reports no observations on chromosome behavior but shows that "parthenogenesis is becoming fixed in *Thalictrum*." In such an instance it seems reasonable to assume that the omission of reduction has become established also. But in *Juglans* the existence of adaptation for wind pollination would indicate that pollination and fertilization are usually essential for seed production and there is experimental proof¹ of this also. Moreover, the extensive researches of Nawaschin and Finn¹² on the cytology of fertilization in *Juglans*, although lacking the treatment of chromosome number and behavior, demonstrate that the process of fertiliza-

tion is typically chalazogamous, there being a well-developed binucleate cell which reaches the embryo sac. Hence the occurrence of reduction may be assumed, in which case embryos must arise through apogamy in the absence of fertilization. But with the haploid number of chromosomes in the somatic tissue of these seedlings, as will be shown below, one might expect even greater deviations from the parent forms than actually occur. Thus, if apogamy and reduction be assumed, it seems necessary also to assume¹³ "the subsequent arrest of the homotypic mitosis (in the cell destined to differentiate the embryo sac) before the division of the nucleus has taken place, resulting in the production of a functional germ cell with a chromosome number double that of its reduced number."

Regarding the other question, the mutations that produce *quercina* seedlings evidently occur only in pistillate *californica* flowers, thus producing seedlings that will not breed true. This indicates that the cytological time of mutation is previous to fertilization.

From her cytological studies of *Oenothera* Lutz¹⁴ has concluded that "all individuals of a given type of vegetative character invariably have identical somatic chromosome numbers regardless of the diversity of origin of the individuals in question," and, further, that "All individuals . . . having a chromosome number much in excess of that in *O. Lamarckiana* displayed certain characters strongly suggesting those of *O. gigas*, chiefly noted in the stoutness of all parts." This suggests that in a form like *quercina*, which is reduced in all vegetative characters, we should expect to find the somatic number of chromosomes to be less than the number typical of the species.

The occurrence of such a mutation in *Juglans* is of especial significance because of the phylogenetic relationships ascribed to these chalazogamous forms. According to Nawaschin and Finn,¹² the preservation of the male cytoplasm in the species of *Juglans* indicates an old tendency inherited from gymnosperm ancestors and furnishes further important proof of the great age of these forms, which stand on the threshold of the angiosperm world. Berry¹⁵ mentions the occurrence of seven species of *Juglans* in the upper Cretaceous deposits, twenty-five in the

Eocene, several in the Oligocene, upwards of twenty in the Miocene, and about twenty-five in the Pliocene, several of the latter being very close to, if not identical with, existing species. These are what remain in the very imperfect geological record. Doubtless many others existed. Now that aggregate mutation is known to have occurred once in such a group, it is reasonable to assume the occurrence of such major discontinuous variations as one of the processes by which new species have been produced. A recent effort¹⁶ to harmonize the older theories of evolution through continuous variation and the modern conception of alternative inheritance assumes that "unit characters" are really composite in nature, but the paleobotanist will not necessarily accept the hypothesis that all new species were built up from such minute discontinuous variations that the effect is one of continuous though gradual change. It is just as reasonable to assume that the fossil species sprang into existence in the same sudden manner as that by which *quercina* made its appearance.

On account of the persistent assumption by some¹⁷ that *quercina* is a natural hybrid between walnut and oak and that the progeny of the original *quercina* trees are composed of walnut and oak seedlings in Mendelian proportions, it should be noted that there is wide variation among the fruiting specimens of the new form as to the proportion of *californica* and *quercina* seedlings they produce. I have tested seeds from three of the original trees distributed by Disher¹ and find they differ very widely in this respect. They certainly produce no oaks and there is no basis for assuming a Mendelian ratio among the progeny.

SUMMARY

1. The belief, which has been held by many, that the new form of walnut, *Juglans californica* var. *quercina*, originated through hybridization between walnut and oak is without foundation in fact. There is no evidence that hybridization with other species of walnut or cross-pollination with other trees of the same species causes the appearance of the new form.

2. Although abnormal, late appearing flowers and fruits occur rather frequently on the California Black Walnut, only one tree has been found to produce abnormal nuts which give rise to *quercina* seedlings, and more than half of the abnormal nuts from this particular tree produce typical *californica* plants. Certainly teratology cannot be considered the cause of the origin of the new variety.

3. The evidence that seedlings of the new variety come from certain apparently normal nuts is conclusive, since a tree has been located that annually produces a small percentage of *quercina* seedlings. Evidently these aberrant individuals are the result of internal changes that take place during the growth of the flowers previous to fertilization. It is possible that evidence of these changes will be discovered by microscopical study of material from tree no. 16 and that further breeding experiments will help to explain them. Meanwhile, whether the true nature of these changes be revealed or not, we know that embryos are produced independently of the influence of self- or cross-pollination, which are capable of developing into individuals possessing characters strikingly different from those of the parent and capable of transmitting those same characters to at least a portion of their progeny. Such occurrences of discontinuous variation are generally recognized as mutations, and the *quercina* walnut is similar to certain mutations in tomato, cotton, tobacco and evening primrose, which have been designated as aggregate mutation.

Transmitted June 15, 1914.

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- ¹³ This is the 5th condition of Grégoire's hypothesis for explaining chromosome numbers in *Oenothera* as set forth by Lutz (cf. 14, p. 426).
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PLATE 13

Juglans californica × *Quercus agrifolia*

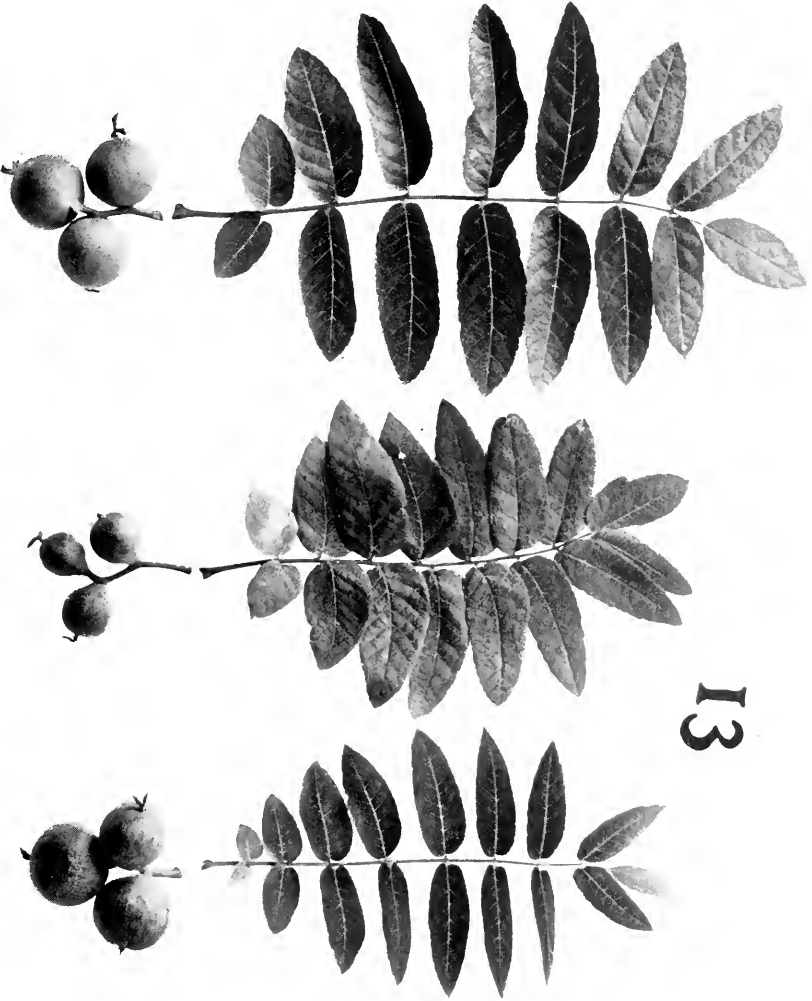
Fig. 1.—*a*, leaf and fruits from F₁ seedling No. 13a.

Fig. 1.—*b*, leaf and fruits from F₁ seedling No. 13b.

Fig. 1.—*c*, leaf and fruits from F₁ seedling No. 13c.

The nuts from which these three F₁ seedlings grew were borne in the same cluster on *J. californica* tree 1 of 1908 hybridization experiments. × 1₂.

13



a

b

c



PLATE 14

Juglans californica × *Quercus agrifolia*

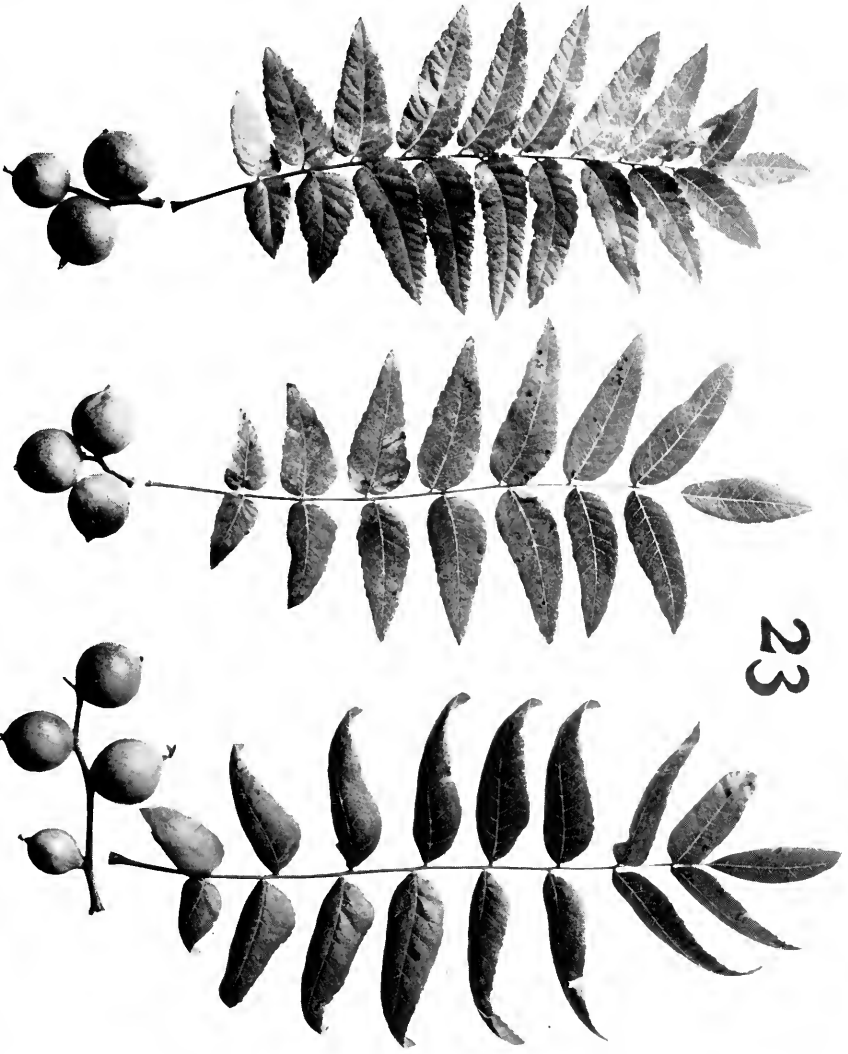
Fig. 2—*a*, leaf and fruits from F₁ seedling No. 23a.

Fig. 2—*b*, leaf and fruits from F₁ seedling No. 23b.

Fig. 2—*c*, leaf and fruits from F₁ seedling No. 23c.

The nuts from which these three F₁ seedlings grew were borne in the same cluster on *J. californica* tree 11 of 1908 hybridization experiments.
× 1/3.

23



a

b

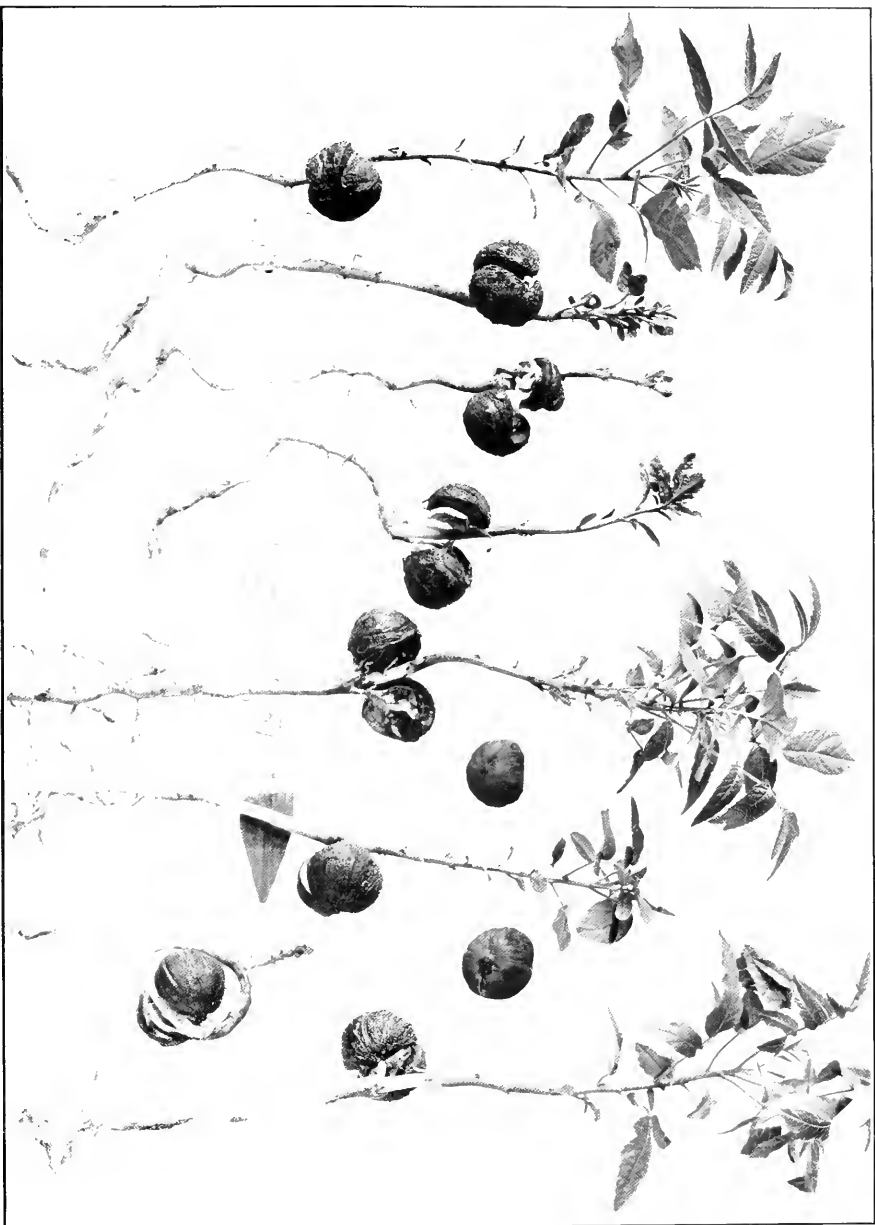
c

PLATE 15

Juglans californica Watson

J. californica var. *quercina* Babcock

Fig. 3.—*a, b, c, d*, typical *californica* seedlings,—*e, f, g, h*, typical *quercina* seedlings. All from the same cluster of abnormal fruits from tree No. 16 in Garden Grove, Calif. $\times \frac{1}{2}$.



a
e
f
b
c
g
h
d

PLATE 16

Juglans californica Watson

J. californica var. *quercina* Babéock

Fig. 4.—*a*, *quercina* and *b*, *californica* seedlings from cluster No. 35 on *J. californica* tree No. 16 in Garden Grove, Calif. × 1.



a

b

PLATE 17

Juglans californica Watson

J. californica var. *quercina* Babcock

Fig. 5.—*a*, *quercina* and *b*, *c*,—*californica* seedlings from cluster No. 97 on *J. californica* tree No. 16 in Garden Grove, Calif. $\times \frac{1}{2}$.



b

a

c

PLATE 18

Juglans californica Watson

J. californica var. *quercina* Babcock

Fig. 6.—*a*, *quercina* and *b*, *c*, *d*,—*californica* seedlings from cluster No. 196 on *J. californica* tree No. 16 in Garden Grove, Calif. $\times \frac{1}{2}$.



PLATE 19

Juglans californica Watson

Fig. 7.—Specimen of polyembryony from cluster No. 208 on *J. californica* tree No. 16 in Garden Grove, Calif. $\times \frac{1}{2}$.

Juglans californica var. *quercina* Babcock

Fig. 8.—Specimen of polyembryony from a cluster of abnormal nuts on *J. californica* tree No. 16 in Garden Grove, Calif. $\times \frac{1}{2}$.



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STUDIES IN JUGLANS, III

(1) FURTHER EVIDENCE THAT THE OAK-LIKE
WALNUT ORIGINATES BY MUTATION

(2) A PARALLEL MUTATION IN *JUGLANS*
HINDSII (JEPSON) SARGENT

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STUDIES IN JUGLANS, III

(1) FURTHER EVIDENCE THAT THE OAK-LIKE
WALNUT ORIGINATES BY MUTATION

BY

ERNEST B. BABCOCK

In previous studies¹ the conclusion was reached that the oak-like walnut, *Juglans californica* var. *quercina* Babcock, was first produced as a result of germinal variation in a tree of the southern California black walnut, *J. californica* Wats., and that the several recurrences of this peculiar walnut resulted from repetitions of the same mutation. It is the purpose of this paper to report further evidence showing that this conclusion was correct at least as regards the origin of the first *quercina* individual, although this same evidence may lead to a different explanation of some of the recurrences of this form.

This evidence consists of the results from hybridizing *quercina* and *californica*. In 1908 pollen from the original, fertile, type individual of *quercina* was applied to several guarded pistillate flowers on a typical *californica* tree. The eleven F₁ seedlings secured are growing on the campus of the University of California. They are all normal *californica* trees. The only evidence of heterosis thus far observed is length of staminate catkins, which is intermediate between the two parents. In 1915 several of these F₁ trees were self-pollinated, or interpollinated, and thirty-six seeds were secured. Up to the present eighteen of these have germinated, producing twelve *californica* and six *quercina*

¹ Babcock, E. B., Studies in Juglans I and II, Univ. Calif. Publ. Agric. Sciences, vol. 2, no. 1, 1913; no. 2, 1914.

seedlings, which is a ratio of 2.67 to 1.33, a deviation from the theoretical monohybrid ratio which might be expected to occur under the laws of chance in 42 per cent of monohybrid populations. While positive statements will not be made until the F_1 trees have been tested on a more extensive scale, yet a valid 3 to 1 ratio even with so few F_2 individuals is certainly an indication that the genetic relationship between *californica* and *quercina* is a difference in a single factor of the same Mendelian reaction system.

The idea that a single factor-difference may so affect the entire chromosome system that the individual is altered more or less in each somatic detail is now generally recognized, yet the direct evidence on which this conception is based is found in a limited number of cases. Morgan² refers to the mutant strains of *Drosophila ampelophila* called "club" and "rudimentary", in which the factor for a certain wing character also conditions the development of certain other morphological and physiological characters. But, thus far in *Drosophila*, no single factor has been found that visibly affects every external feature of the organism. The well-known dwarf or cupid sweet pea is a striking example of the manifold effects of a single factor. This variety differs from the ordinary climbing form of *Lathyrus odoratus* not only in its extremely dwarf stature but also in color of foliage, length of internodes, size and arrangement of flowers, time of anthesis, fertility and viability. Yet it is certain that the variety differs from the species in a single genetic factor. Probably this is as striking a case as has been reported previously, yet in such a conspicuous character as leaf-shape the dwarf variety very closely resembles the species type. Now the oak-like walnut differs from the species type in every gross external feature—shape of leaves, color of foliage, color of bark, habit of growth, structure of inflorescence, structure of flowers, size and structure of fruits, as well as in fertility and viability. Therefore, if tests that are now being made confirm our inference that *quercina* differs from the species in a single genetic factor, it will be a most striking example of the manifold effects of a single genetic factor. Further-

² Morgan, T. H., Mechanism of Mendelian Heredity, p. 209ff., 1915.

more, the demonstration of such a genetic relation between *quercina* and *californica* must be accepted as ample proof that the first *quercina* individual, at least, originated by mutation.

Since the mutant factor is recessive to its normal allelomorph, it is highly probable that *californica* trees which are known to produce *quercina* seedlings are not to be considered as mutating individuals. Such a tree is *J. californica*, "Garden Grove No. 16", which was referred to in the preceding number of this series. This tree may be either the result of a mutation in one of the gametes that produced it, in which case it would, of course, be heterozygous, or it may be a heterozygote produced by hybridization between *quercina* and *californica*. The same is true of the other two *californica* trees known to have produced *quercina*. But the genetic relation between the variety and the species shows that the first production of *quercina* at least must have been caused by a mutation in one genetic factor and that this change occurred in all probability in a germ cell of the grandparent of the first *quercina* tree.

A question arises as to the interpretation of the test of the particular *californica* tree, Garden Grove, No. 16, reported in the preceding paper. In 1913 the unguarded seed from this tree produced about 5 per cent of *quercina* seedlings. The fact that the seed was not self-pollinated under control and so may have been crossed with nearby trees may serve as an explanation of this result. However, another explanation is indicated. This tree is very late in developing its pistillate flowers, the stigmas becoming conspicuous after most other trees have shed their pollen and after this tree has shed much of its own pollen. Now if only a portion of its pistillate flowers are self-pollinated the remainder probably develop apogamously. That would account for fewer *quercina* seedlings than would be expected from self-pollinated seeds in case this tree is heterozygous for the *quercina* factor. For only one-fourth of the self-pollinated nuts would produce *quercina* seedlings, whereas all apogamous seeds would produce *californica* individuals. Presumably all such apogamic plants would contain the diploid number of chromosomes. Hence they would be heterozygous like their parent and for this reason they would be *californica* in type.

There is also the remote possibility that unfertilized pistillate flowers might develop parthenogenetically, the egg-nucleus, containing the haploid number of chromosomes, developing spontaneously into an embryo. In order that such seeds should produce only *californica* individuals it would be necessary that a single dose of the *quercina* factor could not condition *quercina* development even in plants having the reduced number of chromosomes. This is at variance with the concept that Mendelian reaction systems depend upon proportional chemical relations such that one dose of a recessive factor would play the same rôle in an individual having a haploid system as two doses of the same factor play in an individual having a diploid system. Therefore, the former is the more reasonable interpretation.

(2) A PARALLEL MUTATION IN *JUGLANS HINDSII*
(JEPSON) SARGENT

In November, 1914, through the courtesy of Farm Adviser F. F. Lyons, my attention was called to a nursery at Modesto, California, where there were several thousand one-year-old seedling walnuts. Here and there among the typical black walnuts I found a number of plants (fifty or more) that closely resembled *J. californica* var. *quercina* except that they were taller than *quercina* seedlings of the same age and the leaves appeared somewhat larger. Through the kindness of the owner, George F. Covell, seven of these seedlings are now growing on the campus of the University of California. These seven and several that were examined at the nursery were found to have come from typical nuts of *J. hindsii*, the northern California black walnut. The trees that produced the nuts which Covell planted in his nursery are also typical of *J. hindsii*, but as they had been grafted to commercial varieties I was unable to secure seeds from them. However these grafted trees are seedlings from four large northern black trees growing near Lodi, California. Several hundred nuts from each of these trees have been germinated and only seedlings typical of *J. hindsii* have been secured. If any one of these trees is repeating the mutation there is no evidence of it in

the immediate progeny. This is what would be expected if the new variety has the same genetic relation to *J. hindsii* as *quercina* has to *J. californica*.

In an earlier paper³ I proposed to designate *quercina* as *J. californica* mut. *quercina* and the *quercina*-like form of *hindsii* as *J. californica* var. *hindsii* mut. *quercina*. However, the recognition of *hindsii* as a species⁴ simplifies the problem and makes it desirable to describe the new mutant from *hindsii* as a variety of that species and to retain *quercina* as a variety of *californica*. The following description is based upon material gathered from several of the seedlings in Covell's nursery in 1914. The seven seedlings growing on the campus of the University of California are cited as cotypes. It should be noted that the variety name has been chosen for the express purpose of emphasizing the fact that the new variety resembles *quercina* in leaf characters.

NEW VARIETY

Juglans hindsii var. *quercinifolia* Babcock

Tree. Bark and leaves strongly walnut-scented. Pits in plates. Twigs, bud scales, and young leaves granular pubescent. Buds few-scaled axillary or superposed. Leaves 1 to 3½ inches long, alternate, exstipulate, mostly compound with three leaflets; terminal leaflet 1½ to 2 times as long as lateral leaflets and ranging from ¾ to 2¼ inches in length, in form ovate or elliptical, obtuse or truncate at the apex, margin irregularly crenate or serrate; lateral leaflets mostly opposite and sessile, sometimes one or both lacking, occasionally one or two extra ones present; petiole equal to or shorter than lateral leaflets; very rarely with unifoliolate leaves. (Cf. plate 20, fig. 1.)

Nursery of George F. Covell, Modesto, Cal., Nov., 1914. Univ. of Calif. Herb. no. 189541. Cotypes on campus of the Univ. of Calif. (Cf. Div. of Genetics nos. 755a to 755g.)

Plate 20, fig. 1 shows a specimen of *quercinifolia* which was supplied by Covell in 1915. A typical *quercina* seedling is shown in plate 20, fig. 3. The relative size of these two seedlings is of no significance as they were not of the same age. In order to

³ Babcock, E. B., Walnut Mutant Investigations, Proc. Nat. Acad., vol. 1, p. 535, Oct., 1915.

⁴ Jepson, W. L., in Smith, R. E., Univ. Calif. Agr. Exp. Sta. Bull. 203, p. 27 (1909). *Juglans californica* Wats. var. *hindsii* Jepson in Bull. So. Cal. Acad. Sci., vol. 7, p. 23 (1908).

emphasize the fact that *hindsii* is distinct from *californica* even in the young seedling stage, a typical seedling of *californica* and one of *hindsii* are shown in plate 21, figures 5 and 6. The differences between the nuts of the two species are clearly shown. The mature trees of the two species are also strikingly distinct, *californica* being always low and shrub-like in habit while *hindsii* is tall and arboreous in form.

These parallel mutations in two distinct species may appear as degressive rather than regressive variations or, in other words, as cases of reversion to a common ancestral form. If both *quercina* and *quercinifolia* resulted from a change in one genetic factor, as seems likely, and both represent a common ancestral form, then it would follow that both *californica* and *hindsii* sprang fully formed from their common ancestor by mutation. Yet both *quercina* and *quercinifolia* show reduction in morphological characters and *quercina* individuals exhibit low fertility. These symptoms would indicate that the mutation is regressive rather than degressive. However, the fact that any walnut varieties originate by mutation is of significance for the student of evolution, because the Juglandaceae are generally considered as one of the older and more stable groups of angiosperms. They are not supposed to be undergoing changes similar to changes that give rise to new types in the younger, less stable groups. That the origin of these two unique walnuts, or of *quercina* at least, cannot be explained on the basis of hybridization is now fully proved. Evidently *quercina* sprang from *californica* rather than *californica* from *quercina*, and it arose as a result of a change in a single genetic factor, i.e., of mutation in the strict sense.

Transmitted May 31, 1916.

PLATE 20

Juglans hindsii var. *quercinifolia* Babcock

Fig. 1. Specimen from Covell's nursery, 1915. Note typical *hindsii* nut, the husk removed to show the smooth surface. $\times \frac{1}{2}$.

Fig. 2. Halves of typical *hindsii* nut. $\times \frac{1}{2}$.

J. californica var. *quercina* Babcock

Fig. 3. Seedling of *J. californica*, "Garden Grove No. 16." Note typical *californica* nut. $\times \frac{1}{2}$.

Fig. 4. Halves of typical *californica* nut. $\times \frac{1}{2}$.

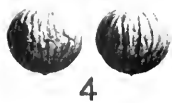
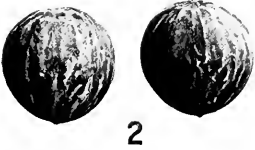
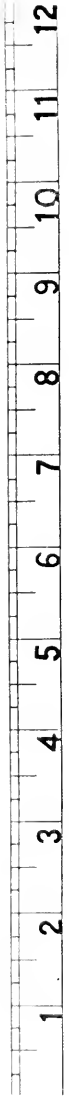


PLATE 21

Juglans californica Watson

Fig. 5. Typical seedling. $\times \frac{1}{2}$.

J. hindsii (Jepson) Sargent

Fig. 6. Typical seedling. $\times \frac{1}{2}$.



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Vol. 2, No. 4, pp. 81-190, plates 22-35

November 24, 1919

MUTATION IN MATTHIOLA

BY
HOWARD B. FROST

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MUTATION IN *MATTHIOLA*¹

BY
HOWARD B. FROST

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INTRODUCTION

It is hardly safe to use the term *mutation* without first defining it. In this paper it will be taken to mean a genotypic change, or a change in essential hereditary constitution, due neither to immediate cross fertilization nor to segregation in a heterozygous parent. No attempt will be made to restrict the term to any of the known or supposed types of such genotypic change; a limitation of this kind, which restricts the generally accepted sense of a widely used term, seems to tend to confusion rather than to clearness.

¹ Paper no. 52, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

DEC 1 7 1919

If we use the term *factor mutation*,² (Babcock, 1918) where the cytological change occurs within a locus, transforming a factor into a different factor, two analogous terms will apply where the cytological change is external to the locus. When the cytological change consists of a loss, reduplication, or transposition of one or more loci it may be called a *locus mutation*, and when the change consists in such phenomena affecting a whole chromosome it may be called a *chromosome mutation*. If the term *mutation* is applied to the cytological change itself, the last two types of mutation may be grouped together as extralocus mutations, while the first type consists of intralocus mutations. Examples of factor mutation are white eye in *Drosophila*, and probably the *rubrinervis* type in *Oenothera*; an example of locus mutation is (possibly) "deficiency" in *Drosophila*; and examples of chromosome mutation are *Oenothera gigas* and *O. lata*.

It is now evident that the immediate problem with *Oenothera* relates to the mechanism of heredity in the genus. There are two sharply opposed views. One is that recently emphasized by Atkinson (1917, p. 254), when he says, "The evidence from *Oenothera* cultures points more and more to the conclusion of Shull that 'a hereditary mechanism must exist in *Oenothera* fundamentally different from that which distributes the Mendelian unit-characters.'" The opposing view is represented by Muller's (1918) strictly Mendelian explanation for *Oenothera*, based on "an *Oenothera*-like case in *Drosophila*"; he says, "The striking parallel between the above behavior and that exhibited in *Oenothera* makes it practically certain that this, too, is a complicated case of balanced lethal factors."

A notable feature of the extensive genetic study of *Oenothera* is the lack of progress toward any definitely supported explanation of its hereditary mechanism which is not Mendelian. The only definite non-Mendelian hypothesis of chromosome behavior so far proposed, aside from "merogony" and other hypotheses (Goldschmidt, 1916) apparently possible but not proved for *Oenothera*, which assume loss of chromatin after fertilization, seems to be Swingle's (1911) "zygotaxis," proposed for the apparently parallel case of *Citrus*. This suggestion that F_1 hybrids may differ, apart from non-uniformity of the P_1 gametes, because of the establishment of permanently different arrangements of the chromosomes in the fertilized egg, still seems to be purely speculative.

With more or less "*Oenothera*-like" cases in other genera, the only definite progress in analysis seems to have resulted from the assump-

² Muller (1918) has recently used *point mutation* in the same sense.

tion of Mendelian segregation. With *Oenothera* itself, the trend of the evidence tends to favor this form of explanation.

This fact is strikingly illustrated by two papers of de Vries (1918, 1919) which have appeared since the present paper was written, especially as Muller's (1918) complete report on the beaded-wing case in *Drosophila* (see especially pp. 471-474, 489, and 498-499) indicates that de Vries had hardly yet realized the full possibilities of the balanced-factor hypothesis. In the light of Muller's masterly demonstration of these possibilities, we may be confident that "mass mutation" is merely ordinary segregation, and that the "unisexual" crosses of *Oenothera* are really "Mendelian" in their essential phenomena. Some difference of usage respecting the inclusiveness of the term *Mendelian* may be involved here, it is true, since apparently de Vries would apply it only to cases where strictly homologous factors are opposed in homologous chromosomes. Since, however (Muller, 1918), there are good reasons for expecting the occurrence of gradations of similarity and of synaptic attraction between opposed loci, and hence of gradations of linkage, the criterion of Mendelian behavior should obviously be the occurrence of segregation between homologous chromosomes, whatever their degree of similarity or amount of crossing over. We have no reason to assume that an "unpaired" factor in a parent would so divide as to be included in *all gametes*; on the other hand, we have learned of a mechanism capable of insuring, in certain particular cases, the inclusion of a certain factor or group of factors either in every *functional* gamete or in every *viable* zygote.

No doubt, as Davis (1917) says, "A great forward step will be taken in *Oenothera* genetics when types of proven purity have been established . . ." Meanwhile, cases of "*Oenothera*-like" heredity in species known to possess the Mendelian mechanism deserve most thorough investigation. Special interest consequently attaches to the peculiar inheritance of certain apparent mutations of the ten-weeks stock (*Matthiola annua* Sweet), a species in which various characteristics are typically Mendelian. A remarkable series of aberrant forms in this species³ has been briefly discussed in two preliminary communications (Frost, 1912 and 1916), and the present paper gives a fuller account of the same phenomena.⁴

³ In the variety "Snowflake," a glabrous, double-producing form with white flowers.

⁴ While this paper was in press Blakeslee and Avery (1919), have reported the occurrence of apparent mutations in *Datura*, which seem to be similar in almost every respect to those here discussed.

Apparent mutants were first found in the course of work on another problem, the relation of temperature to variation (Frost, 1911), conducted at Cornell University. Studied incidentally at first, these new forms were later given special attention. About nine thousand plants, of which about two thousand were progeny of mutant-type parents of peculiar heredity (nearly one-fourth of the latter representing crosses with Snowflake), have been examined altogether. Some of these plants have been grown at Riverside, where hybridization studies with mutant types are in progress. The present account considers the origin and characteristics of these types, their inheritance with self pollination, and the rather meager available data relating to their behavior in crossing.

In connection with the work at Cornell, special acknowledgment is due to the late Professor John Craig, and to Dr. H. J. Webber and Dr. H. H. Love. Facilities for work were furnished by the departments of Horticulture and Plant Breeding of the New York State College of Agriculture.

GENETIC LITERATURE RELATING TO *MATTHIOLA*

The work of Correns (1900) on *Matthiola* furnished one of the earliest confirmations of Mendel's law, and also pointed to complications not found by Mendel. The earlier literature, according to Correns, gives no indication of the study of *Matthiola* hybrids beyond the first generation.

In his later paper on aberrant hybrid ratios, the same author (1902) discusses complications in maize and in *Matthiola*. After referring the deviations found in maize to selective pollination, he considers a suggestion of de Vries relating to environmental modification of Mendelian ratios, and himself suggests the possibility of selective elimination of gametes. He says (pp. 171-172), "Solche Einflüsse brauchten nicht alle Sorten Keimzellen des Bastardes gleichmässig zu treffen, sondern sie könnten eine Sorte stärker angreifen als die andere."

Von Tschermak (1904, 1912) has made extensive studies of *Matthiola* hybrids, considering mainly, as did Correns, pubescence and flower color. The latter of these papers on hybrids in the genera *Matthiola*, *Pisum*, and *Phaseolus* represents a careful analytical test of the "factor hypothesis" of segregating inheritance, leading to the conclusion that the applicability of this hypothesis is strongly con-

firmed by the results secured. This work, with that of Miss Saunders, leaves no possibility of doubt that the typical Mendelian mechanism is present in *Matthiola*.

The most extensive genetic work on *Matthiola* is evidently that of Miss Saunders, reported by herself (1911, 1911*a*, 1913, 1913*a*, 1915, 1916) and by Bateson and Saunders, with others (1902, 1905, 1906, 1908). This also is work on heredity in hybrids, with special emphasis on the factorial interpretation of the various complications relating to pubescence and to "doubleness" of flowers.

Goldschmidt (1913) has explained the inheritance of doubleness by sex linkage and lethal action of a femaleness factor in pollen formation, and his interpretation has been criticized by Miss Saunders (1913). I (Frost, 1915) have presented a somewhat different lethal-factor scheme, and Miss Saunders (1916) has since restated her views and criticized mine.

Muller (1917) has cited the inheritance of doubleness as a case of "balanced factors," in apparent agreement with my formulation.

Apparently no one but the present writer (Frost, 1912, 1916; see also review by Bartlett, 1917) has reported experimental evidence of any notable tendency to apparent mutation in the genus, although de Vries (1906, p. 338) mentions the occasional occurrence of vigorous, rigidly upright individuals (a *gigas* type?), known at Erfurt as "generals," and refers to the rare mutative occurrence of single flowers on branches of double-flowering plants. Doubleness, and color variations in considerable number, have evidently arisen under cultivation, probably through mutative changes.

METHODS

The general cultural methods employed for the first three generations have been very briefly described elsewhere (Frost, 1911).

The plants of the first four years were grown in pots in the greenhouse. The plants of the first generation came from one or both of two packets of commercial seed planted in the fall of 1906, and all plants in the later cultures (possibly excepting series 18) were descendants of these. The cultures will in general be designated by the year in which the seed was sown; the field and greenhouse cultures of 1911 are indicated by 1911F and 1911H respectively.

Part of the seed planted, especially in 1908, came from unguarded flowers. The seed lots where this occurred will be indicated in the

tabulation of parental data by italic figures, while protection possibly defective will be indicated by an asterisk. It is not probable that much vicinism occurred in the greenhouse cultures, since this plant is well adapted to self fertilization.

In the first year's (1906) cultures the plants in each experimental environment were separately numbered. Each plant was designated by its number preceded by two letters indicative of the environment. For greenhouse temperature these letters were C (cool), M (medium temperature), and W (warm); for potting soil⁵ they were S (sand), L (unfertilized "loam"), and G ("good" soil, fertilized). Thus CS1 CS2, WG9, etc., were pedigree numbers of the first generation, and CG2-M8 and WG9-C10 of the second generation. A few syncytyle-donous plants outside the regular cultures of 1907 were called WG9-syn1, etc.

For the work at Riverside a new system of numbering was adopted, better suited to ordinary pedigree cultures, and the numbers from this system are used below in the individual treatment of all but one of the mutant types ("early"). This is essentially Webber's (1906, p. 308) system, except that each initial or P₁ individual of a series is indicated by a letter; a full description has been published (Frost, 1917). With *Matthiola* each type or cross between two types that is tested receives a series number, the apparent mutants themselves always being taken as the initial individuals of their selfed series.

The cultures of 1908 included progeny of various parents, one being WG9-C10, an early and few-noded plant suspected of being a mutant.

The cultures of 1910 consisted of a second-generation test of WG9-C10, and a first-generation test of other possible mutants, with control lots. The plants were all grown on one bench in one greenhouse (house C), from thirty lots of fifteen seeds each, lots 1-17 relating to WG9-C10. The parents descended from WG9-C10 (see table 7) were selected as those with fewest internodes, a medium number of internodes, and most⁶ internodes in each house of the 1908 cultures, earliness of flowering being considered when parents were alike in number of internodes. The control parents were both few-noded and many-noded, relatively to their sibs.

In 1911 eighty progeny lots were grown in the field at Ithaca. Lots 1 to 28, transplanted from the greenhouse, paralleled the test of

⁵ Soil experimentally varied only in the 1906 cultures, temperature varied in the two following years also.

⁶ For house M, not the highest, which was exceptionally high, but the next to the highest.

WG9-C10 made in 1910-11; all available progeny of WG9-C10, except the erenate-leaved apparent mutant WG9-C10-C10, were tested, with check lots between as before. Soil differences and unavoidable differences between lots in time of transplanting combined with hot weather and drought to reduce the value of the results. The remaining fifty-two lots, all field-sown, included a further test of the heredity of aberrant types other than early. Most of these lots, however, were progeny of Snowflake parents, grown to obtain evidence on the relation of temperature to mutation and on the inheritance of doubleness of flowers, and therefore the results are not reported here.

The 1911H cultures constituted a coldframe and greenhouse progeny test of mutant types, mainly in the second generation, the plants being grown in flats.

There was added in 1912-13 a small greenhouse test bearing on the supposed mutative origin of WG9-C10, in view of the apparent possibility that WS1 or WL10, in the same house with the unbagged WG9, might have been heterozygous for the early type—cross pollination then giving the apparent mutant.

Further progeny tests of the mutant types have been made in the field at Riverside, beginning in the fall of 1913. Mainly on account of the unsuitability of the usually hot and dry climate of Riverside, the cultures have been largely experimental and always on a small scale, and germination or development has sometimes been unsatisfactory. Cultures have been started in October, November, January, and February, and a trial culture in progress at the time of writing was started in August. Some of the plants of the 1915-16 cultures were kept until the summer of 1917, and many of them flowered for the first time when about a year old.

In the cultures of 1913, growth was largely unsatisfactory, and with part of the plants aphid injury interfered more or less with the classification of types. In the cultures of 1914, the seeds were largely lost through toxic effects favored by very shallow planting (as at Ithaca) and strong evaporation from the soil. In subsequent planting, the seeds, planted singly in small paper pots, were dropped into relatively deep holes punched in the soil, and covered with sand.

The only field-grown plants closely resembling those grown in the greenhouse at Ithaca, it may be noted, have been those of the 1917 cultures, grown in a lathhouse with added shade from muslin.

In the cultures of 1915-16, with partial shade and more frequent irrigation than before, development was in general good; but even

TABLE 1
Cultures of 1908. Aberrant types: occurrence among progeny of Snowflake parents.

Percentage	Progeny										All apparent mutants	
	Selection	Total number	Smooth-leaved type	Crenate-leaved type	Semi-crenate-leaved type	Narrow-leaved type	Slender type	Leaf-tips misshapen: very late	Leaves numerous and small: very late	Petals spatulate: very late	Number	Percent
CG2	None	85	3	1	1	4
WS1	None	38	1	1	3
WL10	None	34	1	1	4
WG9	None	117	2	1	4
All above	None	274	5 ^b	3	1	2	1	1	1	15	5.47 ± .93 ^c
CG2-F ₁	Selection in notebook ^a	208	1	1	2	.96 ± 1.06
and WG9-F ₁	Selection at trans-planting	440	1	2 ^c	4	.91 ± .73

^a By including all potted plants in each arranged progeny lot (see "Methods," p. 89) up to the first that probably would have been rejected from a selected lot at transplanting.

^b Another, a tricotyledonous seedling of CG2, occurred among unpotted plants.

^c Two others occurred among 11 syneotyis potted ("extra" plants); parents, CG2-W1 and CG2-W9.

^d Another among unpotted plants (parent, WG9-W2).

^e The percentage for this unselected lot is used as *p* for all three probable errors. See p. 145 for the general method of calculation, etc.

here the mutant types, with one exception, often failed to grow satisfactorily or to set seed. Infection, probably by *Fusarium*, evidently was the cause of the death of many of these plants in their second season.

With all cultures grown after (probably) those of 1906, special care was taken to secure random samples of seed, and after 1908 no plants were rejected. The only exception to this statement is the rejection of one pot out of every fifteen, by number and systematically, in the first twenty progeny lots of 1910. For the earlier cultures, a certain amount of selection must be recorded, as follows. In 1906 the small and the largest plants were omitted at potting, and probably any weak and abnormal seedlings had been omitted at the preceding transplanting. In 1907 all markedly weak, late, or abnormal seedlings, as determined mainly by the appearance of the cotyledons, were omitted at the first transplanting; and the same was done in 1908, except that certain lots from old seed were unselected.⁷

These last lots were arranged at transplanting in such a way that the weak and abnormal plants came at the end in each lot.

EXPERIMENTAL DATA

THE OCCURRENCE OF APPARENT MUTANTS

In the cultures of 1906, 88 plants were grown to maturity, none of these being suspected of mutation. In the cultures of 1907, among 170 plants one striking variant appeared; this plant, WG9-C10, was exceptionally small and early in blooming.

In the cultures of 1908, 714 plants were available, including apparent mutants in several hereditary lines as indicated in table 1. A striking feature of the results is the scarcity of apparent mutants among the seedlings classed as strictly normal at transplanting; probably the scarcity in the preceding years was due mainly to the rejection of abnormal seedlings (see "Methods"). The first, second, and fourth of these forms have been common in later cultures, while the third and fifth have been rarer; the last three, if seen at all elsewhere, have not being recognized as belonging to the same types as these three plants.

⁷ One tiny plant from WG9, probably not viable, was discarded.

Table 2 shows the numbers and percentages of apparent mutants found in the cultures of 1910 and 1911F. Since the early type seems to differ from Snowflake only in size and earliness, and is probably inherited without special complications, the available progeny of early-type parents are included in the totals. The progeny of all parents recognized as belonging to other aberrant types are omitted. The second column under "Percentage of mutants" omits doubtful types and individuals, but includes some individuals for which some doubt was indicated in the original records. One rare type of 1911, large-

TABLE 2

Aberrant types: occurrence among progeny of Snowflake and early parents. Apparent selective elimination at or after germination in field-sown cultures.^a

Cultures	Progeny examined ^b	Percentage of apparent mutants	
		All counted	Doubtful omitted
Greenhouse, 1910	338	5.03 ± .82 ^c	4.14 ± .77
Field, 1911, seed house-sown	2072	5.31 ± .33	4.63 ± .31
All above	2410	5.27 ± .31	4.56 ± .29
Same, Snowflake parents only	1364	4.33 ± .41	3.74 ± .38
Field, 1911, seed field-sown (parents all Snowflake)	3927	2.34 ± .24	1.55 ± .22

^a Germination in greenhouse-sown lots, counting only plants examined for type, 93.2 per cent; in field-sown lots, 45.1 per cent.

^b Including some plants of uncertain type, indicated for some lots (when apparently not Snowflake) in tables 1 and 3.

^c For the calculation of these probable errors the percentages on the third line are used as p .

leaved, here omitted, has proved to be genetic, but its determination in these cultures was in general uncertain. A stricter criterion for the second column, elimination of all individuals not considered positively determined, was used in the calculations for the tables for the inheritance of the separate mutant types.

Evidently the more rigorous field conditions of 1911 eliminated many of the "mutants" at or soon after germination. The "coefficient of mutability" with good germination, as was the case with the unselected cultures of 1908, seems to be near 5 per cent, a surprisingly high figure if immediate true mutation is responsible.

Before the aberrant types are considered separately, we may examine (table 3) a detailed illustration of their occurrence in larger cultures. It seems probable, from this evidence, that any descendant of WG9 was capable of producing any of the mutant types so far

TABLE 3
 1911, field; plants transplanted from greenhouse. Aberrant types: occurrence among progeny of Snowflake and early parents^a

Field lot	Ancestry			Total progeny of determinable type	Progeny evidently belonging to aberrant types														All types				
	Generation 1	Generation 2	Generation 3		Smooth-leaved	Small-smooth-leaved	Crenate-leaved	Semi-crenate-leaved	Pointed-crenate-leaved	Medium-smooth-leaved	Narrow-leaved	Narrow-dark-leaved	Slender	Small-convex-leaved	Compact	Curly-leaved	Pointed-light-leaved	Large-leaved	Medium-large-leaved	Large-thick-leaved	Small stout-capsuled	Jagged-leaved	All counted
1			C8	77	1																	3	3
2			C10	70	1																	2	1
7			W18	77	1																	2	4
8			W24	75	1			1														2	5
15			C3	78																		2	2
16			C7	75	1																	3	3
27			W10	73																		3	3
28			W24	78	1																	4	4
26				59	1				1													3	3
3			C2	79	3																	5	5
4			C5	78	2																	6	6
5			C8	78																		1	1
6			C1	76																		2	2
9			WCG9	76	2																	4	4
10			M19	78																		2	2
11			M16	77	1																	4	4
12			M12	77	1																	4	4
13			M17	80	2																	4	4
14			M8	70																		6	6
17			W6	70	1																	5	5
18			W4	65	1																	3	3
19			W11	76																		0	0
20			W9	72																		2	2
21			W5	75	1																	3	3
22			W8	74	1																	5	5
23			W7	72	2																	3	3
24			W3	71	1																	4	4
25			W10	66																		0	0
All parents—totals				2072	27	9	16	4	1	1	20	10	3	2	4	1	3	1	3	3	1	110	96
All parents—percentages				100	1.30	.43	.77	.19	.05	.05	.97	.48	.14	.10	.19	.05	.14	.05	.14	.14	.05	5.31	4.63

^a Individuals with “?” are those considered to be possibly Snowflake. A few others are somewhat uncertain as to type, though evidently not Snowflake. Possibly some of the rarest types should be merged with others. One plant in lot 28, observed before the first regular examination for type as apparently dead and of crenate-leaved type, was omitted.

discovered; the occurrence of the various types suggests a random distribution among the progeny lots. This conclusion is confirmed, and extended to CG2, by the field-sown lots of 1911.

Various parents belonging to mutant types have given other mutant types among their progeny. There is some reason, as table 4 indicates, to suppose that parents of the early type have a more marked tendency to produce these other types than have Snowflake parents.⁸

TABLE 4

1910 and 1911F; sown in greenhouse. Apparent mutants among descendants of WG9-C10 and other ancestors, comparing early parents (pure or heterozygous) with Snowflake parents.

Ancestry	Type of parent	Progeny		
		Total examined	Percentage of mutants	
			All counted	Doubtful omitted
WG9-C10	Early	1046	6.50 ± .47 ^a	5.64 ± .44
Pure Snowflake	Snowflake	558	4.30 ± .64	3.41 ± .60
	Snowflake	806	4.34 ± .53	3.97 ± .50
Both	Snowflake	1364	4.33 ± .41	3.74 ± .38
Both	Both	2410	5.27 ± .31	4.56 ± .29

^a For the calculation of these probable errors the percentages on the last line are used as p .

CHARACTERISTICS AND HEREDITY OF MUTANT TYPES

1. THE EARLY TYPE

So far as is known, WG9-C10 (figs. 1, 2) was the only apparent mutant of the early type in the cultures. Since, however, this type visibly differs from Snowflake only or mainly in quantitative characters, it cannot be positively identified without comparative progeny tests, and therefore may have been represented by mutant individuals not used as parents. WG9-C10 was much smaller proportionately than were its progeny; this difference was probably due to an embryonic abnormality, early blind termination of the main axis, which was occasionally observed elsewhere and probably occurred in this case. Plants of this type, as compared with Snowflake, are, in general, fewer-noded, smaller, and earlier in blooming.

The principal data from the cultures of 1908 are shown in tables 5 and 6, which also indicate the later conclusions as to the segregation of the early type in the cultures of this year; figures 3 and 4 illustrate

⁸ Inspection of the data in detail indicates that this difference is not due to the possible tendency in parents grown in the warm house to more frequent apparent mutation.

TABLE 5

Cultures of 1908. Time from sowing to emergence of corolla of earliest flower of primary cluster. Frequency distributions.^a

Parents:	Singles						Doubles					
	House C		House M		House W		House C		House M		House W	
	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest
<i>Days</i> ^b												
110	1†
111	1†
112
113
114
115
116	1	1
117	1†
118	1†	2
119	3
120	1†	4
121	1†	2	1	1
122	3	2
123	8	1†	1	1	2
124	1	4	1	2
125	7	1†	5	1	3
126	16	7	7
127	3	1†	2	9	2
128	4	3†	8	5
129	7	1†	4	8	7
130	1	1†	3	12†	4
131	1	2	4	2	3
132	1†	1	2	7	8
133	1††	1	1	4	7
134	1	2	2	1	2	7
135	4	4	4	6	6
136	1	1	1	3	1	4
137	7	2	3	3	1	2
138	10	1	9	4	1	1
139	18†	1	1	2	8	1	3
140	1	7	1†	4	13	3
141	10	1	9	1	3
142	4	6	15	1	1
143	4	1	5	2	10	2
144	4	2	9	2
145	3	6	3
146	1	5	2
147	4
148	2	1	1
149	2	1
150	1	1	2
151	1	1	1
152	2
153	1	1
154
155	1	1	1

^a Daggers (†) indicate the position and number of apparent mutants. Double daggers (††) indicate inheritance of parental type (here, early); all *single* progeny of WG9-C10 here reported have been tested for inheritance of this type. The conventional statistical constants corresponding to the house totals of tables 5 and 6 have been published (Frost, 1911); the means for flowering given there are too high by one half-day.

^b To time of observation (upper limit of one-day class).

TABLE 5. CULTURES OF 1908 (Continued)

Parents:	Singles						Doubles					
	House C		House M		House W		House C		House M		House W	
	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest
<i>D. v. s.</i> ^b												
156
157
158
159
160
161
162	1
163
164
165
166
167	1†
168
169
170
171
172
173	1†

^a Daggers (†) indicate the position and number of apparent mutants. Double daggers (‡) indicate inheritance of parental type (here, early); all *single* progeny of WG9-C10 here reported have been tested for inheritance of this type. The conventional statistical constants corresponding to the house totals of tables 5 and 6 have been published (Frost, 1911); the means for flowering given there are too high by one half-day.

^b To time of observation (upper limit of one-day class).

the difference in earliness between the early and Snowflake types. The parents grouped under "rest" include CG2 and WG9 themselves, with four progeny of the former and eight of the latter. Of these fourteen parents, not one has produced exceptionally few-noded progeny like those of WG9-C10.

Apparently WG9-C10 was heterozygous for a "few-nodedness" factor not carried by any of the other parents tested. Neither in the 1907 cultures nor in the 1908 cultures now under consideration did the data suggest that WG9 itself was similarly heterozygous. Tables 5 and 6 include the first 30 progeny of WG9, for each house, as arranged at the first transplanting,⁹ 88 plants altogether; including the remaining plants, mainly weak or abnormal at transplanting, the total is 116. One of the F₂ plants (WG9-syn3-M10) was very suggestive of the early type, but (tables 12 and 13) it gave only Snowflake progeny in a small test.

⁹ See page 89. Two plants not producing a normal main inflorescence are omitted.

TABLE 6

Cultures of 1908. Number of main-stem internodes below first flower-bearing node. Frequency distributions.^a

Parents:	Singles						Doubles					
	House C		House M		House W		House C		House M		House W	
	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest	WG9-C10	Rest
<i>Internodes</i>												
16	1†
17
18
19	1††
20	1†
21
22	2††
23	1†
24
25	1	2†	1†	1	2	9
26	25
27	1	7	...	1	1	29	...	1
28	...	17	6	...	2†
29	...	24	...	1	9	1	...
30	...	13	...	1	14	22
31	...	8	...	2	27	...	1	...
32	...	2	...	7	5
33	...	1	...	15	1†	3
34	1	16	4	...	1	...
35	...	2	...	19	1†	5	...
36	4	1†	1	...	6	...
37	1	1†	8	...
38	8	5	...
39	3	13	...
40	1	6	...
41	1	1†	8	...
42	...	1†	1†	6	...
43	1†	12	...
44	9	...
45	1†	6	...
46	4	3	...
47	3	3	...
48	4
49	1	3	1	...
50	6
51	1	3	24†	...
52	10	1	...
53	6
54	2	2	...
55	1	3
56	7	1	...
57	8
58	1
59	1	1	...
60	3
61
62	1

^a See note a to table 5.

The differentiation of the early race is very marked; with the singles, in fact, the later cultures indicate no case of overlapping in the 1908 cultures, in either character, between extracted pure Snowflake and pure or heterozygous early. The total sterility of the doubles necessarily leaves their constitution somewhat in doubt.

The cultures of 1908 so far suggest that WG9-C10 was a mutant. To be reasonably certain, however, we must have further evidence (1) on the fact and mode of inheritance of the supposed new type, and (2) on the possibility that either WG9 or some other plant of the cultures of 1906 brought the character into the cultures. We shall now consider somewhat extensive evidence bearing on these points, concluding with a special test of the possibility of vicinism.

When I last saw the warm-house plants of 1906, three were known to be singles, and all but two of the rest were recorded as certainly or probably doubles. Seed was secured from these three singles only, and presumably no other singles occurred in the house. Since this seed was all from unguarded flowers, we must consider the possibility that WS1 or WL10, the other warm-house singles, brought the early factor into the cultures. It is also barely possible that pollen was brought to WG9 from some plant not in this greenhouse.

These two parents were tested in supplementary cultures, in house C in 1907, and in house W in 1908. The 1907 progeny averaged slightly earlier than those of other parents, but this may have been due to their position, which was much nearer a partition separating the house¹⁹ from a warm greenhouse. Unfortunately the internodes were not recorded.

In the 1908 cultures these lots were potted two days later than most of the other lots and one day later than the WG9 lot, and for some unknown reason the WL10 lot wilted badly for some days. The parents in question gave singles (16 and 11 plants respectively) which when compared with progeny of CG2 and WG9 (23 and 15) might suggest that the parents were heterozygous for the early type. The results with the similar numbers of doubles decidedly disagree with these, and suggest that cultural accidents produced the differences; the WS1 lot was not exceptional, while all the WL10 progeny were grouped near the lower end of the range of the other lots. In view of all the facts, the data hardly deserve tabular presentation, but they raise a question requiring further study; a later test is reported below.

¹⁹ A temporary substitute for the regular house C.

1910, greenhouse. Ancestral data and numbers of progeny available for frequency-polygon constants.

TABLE 7

Lot	Ancestry ^a			Parental internodes		Numbers of progeny ^b	
	Generation 1	Generation 2	Generation 3	Parent-lot mean	Parental value	Single	Double
1		C1 (Snowflake)	M3 (Snowflake)	32.4	35	7	6
2			M9 (Snowflake)		30	9	5
3			C2 (Early)		16	8	6
4		C10 (Early)	C5 (Early)	21.4	20	8	6
5			C1 (Snowflake)		27	7	7
6		C5 (Snowflake)	M6 (Snowflake)	36.0	33	7	7
7			M9 (Snowflake)		39	4	10
8			M4 (Early)		21	6	8
9			M2 (Early)		21	6	8
10	WCG9 (Snowflake)	C10 (Early)	M7 (Snowflake)	27.3	25	6	8
11			M3 (Snowflake)		34	3	10
12		C9 (Snowflake)	C3 (Snowflake)	28.5	27	9	5
13			C7 (Snowflake)		29	5	8
14		C10 (Early)	W6 (Early)		33	8	4
15			W5 (Early)		42	7	7
16			W10 (Snowflake)		55	5	8
17		C9 (Snowflake)	M5 (Snowflake)	34.0	38	6	8
18			M8 (Snowflake)		33	7	5
19	CG2 (Snowflake)	C2 (Snowflake)	C6 (Snowflake)	27.25	27	4	10
20		W4 (Snowflake)	W3 (Snowflake)	54.6	54	6	4
21			C17 (Snowflake)	?	?	7	6
22		syn3 (Snowflake)	M10 (Snowflake)		25	7	6
23			M11 (Snowflake)		33	6	7
24	WCG9 (Snowflake)	C:28 (Smooth-leaved)		30.4	42	7	7
25		W2 (Snowflake)	M7 (Smooth-leaved)	36.2	41	4	7
26		W2 (Early)	C10 (Crenate-leaved)	21.4	19	3	10
27		W2 (Snowflake)	W2 (Crenate-leaved)	47.5	43	6	9
28	WS1 (Snowflake)	W7 (Snowflake)	C5 (Slender)	29.8	25	3	12
29		W:16 ('Snowflake')		49.1	38	7	7
30	WLI10 (Snowflake)	W:25 ('Bushy')			34	3	8
		W:20 ('Late, many-noded')		48.6	73	9	1

^a Seed from bagged flowers except with lots 20 and 23. Parental type determined partly by the progeny tests to be reported. The last two parents gave only Snowflake progeny, aside from one apparent mutant from the former parent.

^b Excluding several plants not producing flowers on the primary axis.

In the cultures of 1910 and 1911F, all the 1908 progeny of WG9-C10 were tested. On account of the variable nature of the quantitative character involved, an elaborate study was necessary. Only small cultures could be grown in the greenhouse; these were supplemented by larger lots in the field in 1911, but inhibition of flowering by the hot summer, together with the effects of disease and soil variations, made the field results erratic and necessitated special methods of treatment of the evidence.

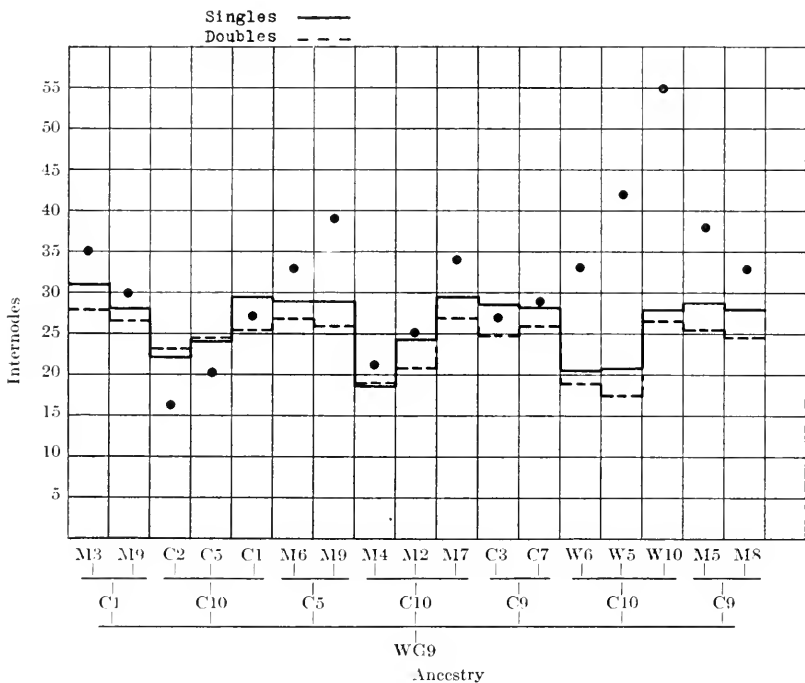


Chart 1. Cultures of 1910. Internodes: parental values and progeny means (respectively shown by dots and lines) for progeny lots 1 to 17, omitting aberrant progeny. Parental values should be compared only for the same house.

Table 7 gives the available data for the parents of the 1910 cultures, and the numbers of progeny available for quantitative data. The order of the pedigree numbers here is the same as that of the progeny lots on the greenhouse bench. For convenience, the 1910 tests of other mutant types, together with tests of several Snowflake parents, are included in the table (lots 18 to 30).

The plants were grown in house C of the previous work. Two or three plants (one shown in fig. 25) were extremely vigorous, presumably because of some accidental soil difference; aside from these, a few apparent mutants, and a few plants otherwise abnormal, the plants were fairly uniform except where heterozygosis was to be expected.

The data for time of flowering, as with the 1908 cultures, show the same main features as the internode data, and only the latter will be considered in detail. The types were again more widely different in internodes than in earliness, a fact which seems to indicate that the early type grows more slowly than Snowflake.

So large and so regular are the differences in internodes that the means of these very small lots seem worthy of presentation (chart 1).¹¹ Apparently the few-noded character was carried, among the nine parents descended from WG9-C10, by all except the three parents having the highest numbers in their respective houses.

Tables 8 and 9 give the internode frequencies for the singles and doubles respectively, by separate progeny lots and by groups of similar ancestry. The range of variation for the check lots, omitting the indicated apparent mutants and other apparently abnormal plants, is rather surprisingly small, as is the case with the cool-house cultures of 1908. The three late progeny of WG9-C10 give lots closely corresponding in range to the check lots, only one individual falling below the range of the combined check lots. The six early and medium progeny of WG9-C10, on the other hand, give distributions of far greater range than do the check parents, extending to much lower values.

Tables 10 and 11 give the ordinary statistical constants for the grouped lots. The mean number of internodes, for both singles and doubles, is about 25 per cent lower in the progeny of the six few-noded parents, the difference being not far from ten times as great as its probable error. The increase in variability with the progeny of the early parents is also striking, and the difference is about five to six times its probable error. With time to flowering, it may be noted, the differences are similar to those with internodes, but somewhat less marked in the case of the mean; the flowering data are not given here.

It is plain that the previous conclusion as to the heterozygous nature of WG9-C10 is sustained. The elimination of the apparent mutants

¹¹ Calculated with the apparent mutants and four other apparently abnormal plants eliminated; see tables 8 and 9.

TABLE 8—(Continued)
 1910, greenhouse. Number of main-stem internodes below first flower-bearing node. Frequency distributions for singles.^a

Ancestry	WG9															CG2, WSL, WL10 & WG9																
	Gen. 1			Gen. 2			Gen. 3			C9			C10			All tested																
	C1			C10			C5			C10			C9			C10			All tested													
Inter-nodes	M3	M9		C2	C5		C1		M6	M9		M4	M2	M7		C3	C7		W6	W5	W10		M5	M8		C10	Not late	Late	Late	Rest	All tested	
35	1A	1†	1
36	2
37	...	1†	2
38	1
39	1
40	1
41	1
42	1
43	1
44	1
45	1
46	1
47	1
48	1
49	1†	2

^a The daggers indicate the position and number of apparent mutants. A indicates diseased, injured, or otherwise abnormal individuals. These indications are omitted from the last two columns.

^b This column includes lots 18 to 30, largely progeny of parents tested as possible mutants; see tables 12 and 13. WG9-C10-C10 belonged to the crenate-leaved type, and its progeny are therefore included in totals only with the last two columns. Plainly it was heterozygous for earliness.

TABLE 10
 1910, greenhouse. Number of main-stem internodes below first flower-bearing node. Constants of the frequency polygon for all plants producing main-stem flower cluster.

Ancestry			Number of progeny	Mean	Standard deviation	Coefficient of variation
Gen. 1	Gen. 2	Gen. 3				
Singles WCG9 All (4) All (4)	Late { C10 Various All	Late { Late Early Various types All	54	29.80 ± .32	3.47 ± .22	11.63 ± .77
			15	29.00 ± .42	2.39 ± .29	8.26 ± 1.02
Doubles WCG9 All (4) All (4)	Late { C10 Various All	Late { Late Early Various types All	43	22.56 ± .61	5.91 ± .43	26.21 ± 2.03
			72	29.47 ± .32	4.01 ± .23	13.61 ± .78
			184	27.91 ± .26	5.22 ± .18	18.70 ± .68
			54	26.50 ± .27	2.95 ± .19	11.15 ± .73
			25	26.44 ± .29	2.14 ± .20	8.08 ± .78
			39	20.92 ± .49	4.55 ± .35	21.74 ± 1.74
			94	25.78 ± .22	3.22 ± .16	12.49 ± .62
			212	25.15 ± .18	3.91 ± .13	15.55 ± .52

TABLE 11
Same as table 10 except for omission of aberrant individuals and mixed groups.^a

	Ancestry			Number of progeny	Mean	Standard deviation	Coefficient of variation
	Gen. 1	Gen. 2	Gen. 3				
Singles {	WG9	{ Late C10	Late	49	28.92 ± .13	1.37 ± .09	4.73 ± .32
			{ Late Early		29.00 ± .42	2.39 ± .29	8.26 ± 1.02
					21.97 ± .49	4.50 ± .35	20.47 ± 1.65
Doubles {	WG9	{ Late C10	Late	53	26.02 ± .16	1.68 ± .11	6.46 ± .43
			{ Late Early		26.50 ± .30	2.16 ± .21	8.15 ± .80
					20.56 ± .47	4.20 ± .33	20.42 ± 1.69

^a See notes to table 8.

and the other abnormal plants presumably gives a better comparison as to mean and variability, but the conclusion is the same in either case. The three many-noded (late) parents descended from WG9-C10 give no definite indication of being genetically different from the "check" lots not descended from WG9-C10, while the variability constants are sufficient, taken alone, to make probable the genetic differentiation of the fewer-noded progeny of WG9-C10. Apparently all the fewer-noded progeny of WG9-C10 that were tested—seven, when WG9-C10-C10, a crenate-leaved apparent mutant (tables 12 and 13), is included—were either simplex or duplex for presence of an earliness factor or factors.

The variability of all the thirty progeny lots, taken together, is high, as might be expected, though decidedly below that of the progeny of early parents. This high variability is due only in very small part to the progeny of the five or six supposedly mutant parents; the last thirteen lots, alone, are much less variable than the mixed early lots. The portion of the cultures containing these progeny lots from aberrant parents was conspicuous for irregularity of germination, and, on the whole, a relatively low rate of germination.

A few of the last thirteen lots give more evidence bearing on the origin of WG9-C10. The early WG9-syn3-M10 (tables 12 and 13) gives no evidence of genotypic differentiation from its ordinary sib, WG9-syn3-M11; WS1-W₂16, another phenotypically early parent, also failed to transmit earliness to its progeny. CG2-C2-C6, on the other hand, although itself an ordinary plant, shows a rather suspicious tendency to the production of early and few-noded progeny, but better evidence would be required for any positive conclusion. WG9-C10-C10 appears, from the data in tables 12 and 13 and from observation of the flowering of plants of the next generation in the 1911H cultures, to have been heterozygous for the early type, as well as for the crenate-leaved type. We find in this test no definite indication that the early type has appeared elsewhere than in WG9-C10 and its descendants.

The F₂ progeny of WG9-C1, an abnormal plant whose F₁ progeny were unusually and uniformly early but not few-noded, have been included with the other check lots without question. This treatment seems justified by the flowering data, which do not indicate any repetition of the precocious development of the first-generation plants; the peculiarities of the F₁ cultures, if not a mere cultural accident, presumably depended on the very abnormal development of the parent,

TABLE 12

1910, greenhouse, lots 18 to 30. Number of main-stem internodes below first flower-bearing node. Frequency distributions for singles.^a

Ancestry	Gen. 1	CG2		WG9							WS1		WL10	
	Gen. 2	C2	W4	syn (M) 3			C ₂ 28	W2	C10	W2	W7	W ₂ 16	W ₂ 25	W ₂ 20
	Gen. 3	C6	W3	C17	M10	M11	M7	C10	W2	C5
<i>Inter-nodes</i>														
21	1	1†
22	1†
23	1†
24	1
25
26	1	1†	2††
27	1	1	1†	1†
28	1	2	1	1†	2	2
29	1	2	1	3
30	1	3	3	2	3
31	1	1
32	1	1
33	1	1†
34	1	1
35
36	1
37
38	1
39	1†
40
41	1†
42
43
44

^a See table 7 and notes to tables 5 and 8.

TABLE 13

Same as table 12, for doubles.^a

Ancestry	Gen. 1	CG2		WG9							WS1		WL10	
	Gen. 2	C2	W4	syn (M) 3			C ₂ 28	W2	C10	W2	W7	W ₂ 16	W ₂ 25	W ₂ 20
	Gen. 3	C6	W3	C17	M10	M11	M7	C10	W2	C5
<i>Inter-nodes</i>														
16	1
17	2
18
19	1
20
21
22	1
23	1	2	3††	1
24	2	1	2
25	4	1	1
26	2	2	1	1
27	2
28	1
29	1
30
31
32
33
34

^a See table 7 and notes to tables 5 and 8.

with its aborted main axis and very late production of a flowering shoot.

Table 14 shows the general plan of the house-sown field cultures of 1911. The progeny of WG9-C10 were arranged as before in the order of their numbers of internodes for each house of the 1908 cultures, beginning with the lowest numbers. The parental values for flowering and internodes are the values indicated by “‡” in tables 5

TABLE 14
1911; field, plants transplanted from greenhouse. Ancestry, seed, and numbers of progeny.^a

Lot	Ancestry			Seeds sown	Number of plants alive 33 days after sowing	Numbers of plants for data on mutation and flowering					
	Gen. 1	Gen. 2	Gen. 3			Total ^b	Singles	Doubles			
1 } 2 }	WG9	C5	{ C8	80	79	77	34	43			
			{ C10	71	71	70	36	34			
3 } 4 }		C10	{	C2	80	80	79	39	39		
				C5	80	79	78	40	38		
				C8	80	79	78	35	43		
				C1	80	78	76	36	40		
5 } 6 }		C5	{	W18	80	79	77	37	40		
				W24	80	76	75	41	34		
7 } 8 }		C10	{	M4	80, <i>26</i>	77	76	30	45		
				M9	80	80	78	36	41		
				M6	80, <i>63</i>	77	77	34	42		
				M2	80, <i>71</i>	78	77	36	40		
				M7	80	80	80	37	42		
				M8	80	74	70	33	37		
9 } 10 }				C9	{	C3	80	78	78	30	48
						C7	80	76	75	32	43
11 } 12 }		C10	{	W6	80	74	70	31	39		
				W4	80, <i>21</i>	70	65	26	38		
				W11	80	78	76	34	42		
				W9	80, <i>19</i>	76	72	24	47		
				W5	80	79	75	33	42		
				W8	80, <i>14</i>	76	74	36	36		
				W7	80	75	72	32	40		
				W3	80	73	71	37	34		
13 } 14 }		C10	{	W10	80	74	73	33	39		
				W24	80	80	78	32	45		
15 } 16 }		C9	{	80	60	59	27	32		
				80	60	59	27	32		
17 } 18 }	C10	{	80	60	59	27	32			
			80	60	59	27	32			
19 } 20 }	C9	{	W10	80	74	73	33	39			
			W24	80	80	78	32	45			
21 } 22 }	C9	{	W10	80	74	73	33	39			
			W24	80	80	78	32	45			
23 } 24 }	C9	{	W10	80	74	73	33	39			
			W24	80	80	78	32	45			
25 } 26 }	C9	{	W10	80	74	73	33	39			
			W24	80	80	78	32	45			
27 } 28 }	C9	{	W10	80	74	73	33	39			
			W24	80	80	78	32	45			

^a For plan of arrangement and parental data, see page 86 and tables 5 and 6. Seeds from unguarded flowers are indicated by italic figures; where two numbers are given the first is the total.

^b Including twelve plants (all late mutants) with which determination of the form of flower was impossible.

and 6, in the order there given, except that the arrangement by internodes reverses the two-day difference in earliness of the parents of lots 19 and 20; for convenient comparison, the parental and parent-lot internode values are included in table 19.

Two progeny lots were set in each of the fourteen rows; probably the soil was less favorable at the east end of the plot, and hence for the even-numbered lots, at least in about the last seven rows out of the fourteen.

The plants were beginning to grow very rapidly when moved to the field. On account of deficient soil moisture and excessive heat, the transplanting was slow and in part purposely delayed, covering a period of five days. Lots 21 to 28 were set three days later than lots

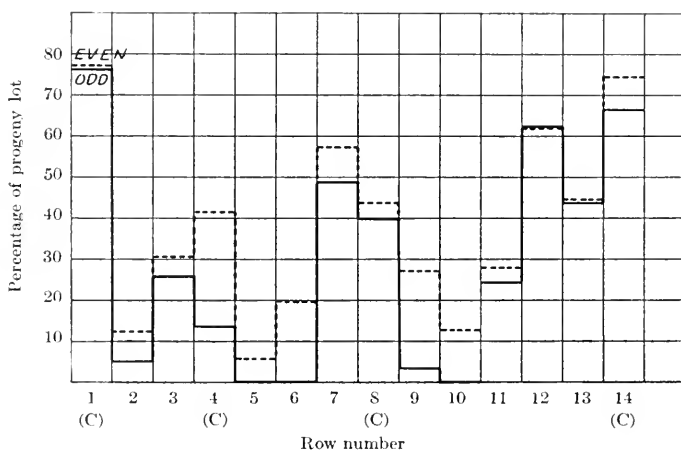


Chart 2. 1911, field; lots transplanted from greenhouse. Percentages of progeny lots not flowering by November 3, for singles. Apparent mutants and injured plants eliminated. Odd-numbered lots represented by solid line. (C) indicate check rows. The curves are broken between rows 10 and 11, where a cultural difference enters.

11 to 20, and the later loss of roots resulting seems, in spite of rain coming the next day, to have seriously delayed flowering. Lots 1 and 2 wilted badly after transplanting, and some difference in soil conditions in the flats, rather than a genetic difference, was doubtless responsible for the exceptional lateness of these lots. Lot 20 lost an exceptionally large leaf area as a result of transplanting. A fungus disease (a slow stem rot) was more common on lots 20 to 24 than elsewhere; it doubtless killed some young plants and delayed or prevented flowering in some other cases. Possibly the soil was poorer in the later rows.

TABLE 15

1911, field; plants transplanted from greenhouse. Plants alive November 3, not having flowered. Singles.

Row	Lot	Non-flowering plants	Non-flowering, Snowflake and early types ^a	Lot	Non-flowering plants	Non-flowering, Snowflake and early types ^a
1	1	27	26	2	29	28 (27)
2	3	5	2	4	7	5
3	5	9	9	6	11	11
4	7	7	5	8	17	17
5	9	0	0	10	2	2 (1)
6	11	1	0	12	9	7
7	13	19	18 (17)	14	20	19
8	15	12	12	16	14	14
9	17	1	1	18	8 (7?)	7 (6?)
10	19	0	0	20	3	3
11	21	8	8	22 ^b	11	10
12	23	21	20	24	23	23
13	25	16	14 (12)	26	14	12
14	27	23	22	28	24	24

^a Omitting non-flowering apparent mutants. For the numbers in parenthesis, "doubtful mutants" are classed as mutants. Two plants accidentally seriously injured, in lots 14 and 25, were counted out with the mutants.

^b The stem-rot disease (see p. 108) was evidently worst in lot 22; some two or three of the worst infected plants (included above) were nearly or quite dead by November 3.

TABLE 16

Same as table 15, for doubles.

Row	Lot	Non-flowering plants	Non-flowering, Snowflake and early types ^a	Lot	Non-flowering plants	Non-flowering, Snowflake and early types ^a
1	1	4	2	2	0	0
2	3	3	1	4	1	1 (0)
3	5	3	3	6	0	0
4	7	3	2 (1)	8	3	1
5	9	2	0	10	0	0
6	11	0	0	12	0	0
7	13	4	2 (1)	14	1	1
8	15	2	2	16	6	5
9	17	1	0	18	3	3 (2)
10	19	0	0	20	2	2
11	21	3	1	22	1	1
12	23	5	5	24	4	4
13	25	1	1	26	7	5
14	27	1	1	28	10	8

^a See notes to table 15.

Altogether, these cultures are doubtless much less reliable for their size than the greenhouse tests of the early type, but they nevertheless, with due consideration of the points just mentioned, seem to permit of fairly safe conclusions for most of the parents.

The plants were examined for flowering every other afternoon from July 4 to November 3, inclusive (73 to 195 days from sowing). A very large part of the plants flowered in July, some in August, and a few still later. Evidently the high summer temperature largely inhibited flowering; many of the singles and a few of the doubles entirely failed to flower.

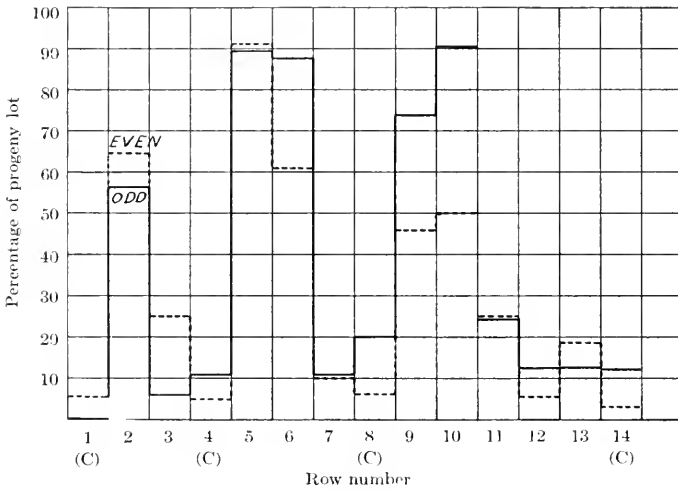


Chart 3. 1911, field; lots transplanted from greenhouse. Percentages of progeny lots with primary cluster flowering or aborted by October 10-16, for singles. Lines as in chart 2.

Figures 5 and 6 show the plants in July. Growth was usually vigorous through the season, but the internodes were very short, the branches numerous, and the region of the terminal inflorescence often abortive, so that determination of the number of main-stem internodes was not practicable. The emergence of the earliest corolla on the plant was recorded at the bi-diurnal observations, and at two periods during the season the aborted primary clusters were noted.

The data show very definitely the transmission of "earliness" by the fewer-noded progeny of WG9-C10. Tables 15 and 16 show the numbers of plants alive, without having flowered, on November 3; the figures are thus a measure of lateness. The two progeny lots in each row are given one line in each of the tables, in order to facilitate separate comparison of the fourteen lots in each end half of the plot.

The last column, with the apparent mutants omitted, no doubt gives the best comparison. The data for the singles, reduced to percentages, are also given in chart 2.

The doubles, which are often earlier to flower than the singles under unfavorable climatic conditions, flowered so generally that table 16 presents no significant differences. The singles (table 15), however, give definite evidence of segregation; the lots in rows 2, 5, 6, and 9 to 11 all show a tendency to early flowering. Lot 26, consisting of F₁

TABLE 17

1911, field; plants transplanted from greenhouse. Singles with primary inflorescence flowering or aborted as indicated.^a

Row	Lot	Aborted by July 29	Flowering or aborted by Oct. 10-16	Lot	Aborted by July 29	Flowering or aborted by Oct. 10-16
1	1	0	0	2	0	0 (2)
2	3	12 (13)	19 (22)	4	20	24 (26)
3	5	2	2	6	4	8 (9)
4	7	2 (3)	3 (4)	8	1	1 (2)
5	9	22	27	10	26	33
6	11	25	29 (30)	12	17	22
7	13	1	2 (4)	14	2 (3)	2 (3)
8	15	4	5 (6)	16	0 (1)	1 (2)
9	17	19 (20)	20 (23)	18	9	12
10	19	25 (27)	29 (31)	20	11	12
11	21	4	6 (8)	22	7	9
12	23	2	3 (4)	24	1	1 (2)
13	25	0	3 (4)	26	3	4 (5)
14	27	1	3 (4)	28	0	0 (1)

^a In this table and also in table 18 the numbers in parenthesis include the probable but somewhat doubtful cases.

progeny of WG9-C10, is decidedly earlier than the adjacent lots. Lot 25 also appears early, however.

Tables 17 and 18 give a direct measure of earliness, relating to the primary inflorescence alone. The clusters visibly aborted were in general relatively far advanced, and those aborted at the earlier date correspond to decidedly early flowering; consequently the flowering and aborted clusters are classed together as early. Chart 3 gives the percentages for singles.

Here the data for the doubles show fairly consistent differences in the number aborted at the earlier date, while the October totals are

less regular. There are contrasts similar to those of table 15 up to lot 26, which is late, while the check lots 27 and 28 are early. The singles show the type differences very strikingly throughout lots 1 to 20, while lots 21, 22, and 26 give less positive indications of the presence of the early factor.

Table 19 gives the numbers of singles flowering, in primary inflorescence or elsewhere, by November 3, when growth had practically stopped. The indications are in general the same as with the data already discussed, with better evidence than usual that lots 21 and 22

TABLE 18
Same as table 17, for doubles.^a

Row	Lot	Aborted by July 29	Flowering or aborted by Oct. 10-16	Lot	Aborted by July 29	Flowering or aborted by Oct. 10-16
1	1	15	30	2	6	22 (23)
2	3	23	33 (34)	4	21	35
3	5	12	29 (30)	6	16	30 (31)
4	7	17	30	8	8	22 (24)
5	9	25	41	10	24	41
6	11	25 (26)	40 (41)	12	23	40
7	13	16	28	14	22	31
8	15	27	37 (39)	16	11 (12)	29 (32)
9	17	20	35	18	20 (21)	32 (33)
10	19	21	41 (42)	20	21	42 (44)
11	21	22	35	22	18	27 (28)
12	23	16 (17)	27 (28)	24	10	16 (17)
13	25	17	25	26	9	15
14	27	21	29 (30)	28	20	25 (27)

^a See note to table 17.

possessed the early factor. The mean time of flowering is irregular, but shows some effect of the earliness factor. Lot 26 is late as to number flowering, but early as to mean.

Table 20, for doubles flowering by August 1, no doubt gives more reliable means; these means disagree with our scheme only in lot 26 and perhaps lot 22.

According to tables 17-20, the fewer-noded check parent of each check row has usually given the earlier progeny. In fact, the agreement of parental and progeny differences, throughout the cultures, is decidedly remarkable. It is unfortunate that the later parents were always placed in the east half of the row, especially in view of the fact that there was indication of important differences in soil and

TABLE 19

1911, field; plants transplanted from greenhouse. Time from sowing to emergence of earliest corolla. Singles.

Row	Parent-lot internode mean	Lot	Parental internode number	Progeny flowering by Nov. 3		Lot	Parental internode number	Progeny flowering by Nov. 3	
				Number	Days to flowering			Number	Days to flowering
1	29.60	1	29	7	147.14	2	32	7	128.57
2	21.40	3	16	34	91.94	4	20	33	105.45
3		5	25	26	119.46	6	27	25	104.08
4	49.57	7	46	30	103.13	8	54	24	105.67
5	27.33	9	21	30	91.73	10	21	34	91.12
6		11	22	33	98.85	12	25	27	108.30
7		13	34	18	100.67	14	41	13	120.62
8	28.50	15	27	17	112.94	16	29	18	118.00
9	42.56 ^a	17	33	30	100.27	18	35	18	109.67
10		19	36	34	97.35	20	37	21	117.81
11		21	42	25	129.36	22	45	25	121.76
12		23	49	11	122.00	24	51	14	151.57
13		25	55	15	136.40	26	13	121.08
14		47.80	27	46	10	159.40	28	56	8

^a This parent-lot value does not apply to lot 26, which consists of progeny of WG9-C10 itself.

TABLE 20

Same as table 19, for doubles flowering by August 1.

Row	Lot	Progeny flowering by Aug. 1 ^a		Lot	Progeny flowering by Aug. 1	
		Number	Days to flowering		Number	Days to flowering
1	1	38	90.26	2	33	91.03
2	3	36	80.22	4	36	80.00
3	5	39	84.46	6	39	84.10
4	7	36	81.28	8	31	84.32
5	9	42	75.86	10	41	76.59
6	11	42	77.90	12	40	80.25
7	13	37	84.32	14	35	84.80
8	15	46	83.87	16	33	85.21
9	17	37	80.43	18	34	84.12
10	19	42	78.24	20	39	83.85
11	21	39	85.95	22	30	87.93
12	23	33	89.03	24	26	88.85
13	25	32	88.56	26	21	89.05
14	27	35	88.97	28	29	90.28

^a Only 48 more doubles altogether flowered by November 3, and 25 of these were in the even-numbered lots 20 to 28.

probably in the incidence of disease, favoring the plants in the west half. The internode data of 1910, however, show a similar tendency. Small genetic differences are suggested, though it would be remarkable if they were so uniformly present in these plants of a single line of a usually selfed species, descendants of parents and a common grand-parent grown under glass.

If such differences exist in the race, conceivably some combination due to crossing might simulate an early mutation. The evidence as a whole, however, does not favor such an origin for our early type; it is widely divergent from the Snowflake type, and seems to depend on a single main factor difference from Snowflake.

TABLE 21
Cultures of 1912. Ancestry and parental data.

Lot	Parent	Parental data			Seeds sown
		Probable type	Days to flowering ^d	Internodes ^d	
1	WS1-W ₂ 16	Snowflake ^a	120.5	38	15
2	WG9-C10-W6	Early	116.5	33	15
3	WL10-W ₂ 2	Snowflake	139.5	51	15
4	WL10-W ₂ 3	Snowflake ^a	120.5	38	15
5	WS1-W ₂ 1	Snowflake	141.5	57	15
6	WL10-W ₂ 14	Snowflake ^a	126.5	38	15
7	WL10-W ₂ 7	Snowflake	145.5	54	15
8	WG9-C10-W8	Early	129.5	45	15
9	WS1-W ₂ 12	Crenate-leaved ^{a,b}	119.5	34	7 ^c

^a Suspected before testing of belonging to the early type; first parent also tested in 1910.

^b A heterozygote between the crenate-leaved and Snowflake types.

^c Probably open pollinated.

^d All the parents grew in the same house at the same time.

The essential feature of the supplementary cultures of 1912, since no seed of WL10 remained, was a test of two pairs of early and late progeny of WL10 (lots 3 and 4, 6 and 7, table 21), in comparison with two control lots—one (lot 2) from a known early parent, descended from WG9-C10, and one (lot 5) from a late descendent of WS1. Incidentally, WS1-W₂16 and WG9-C10-W8 were retested, and the few available seeds of WS1-W₂12 were used to test that phenotypically early parent.

The results are given in tables 22 and 23 and chart 4. The very low individual from WS1-W₂16 came from a very weak embryo, and should be disregarded; the exceptionally high general range of this lot, which was also visibly behind all others in development, was prob-

TABLE 22

Cultures of 1912. Number of main-stem internodes below first flower-bearing node. Frequency distributions for singles.^a

Ancestry	Gen. 1	WS1	WG9	WL10		WS1	WL10		WG9	WS1
	Gen. 2	W ₂ 16	C10	W ₂ 2	W ₂ 3	W ₂ 1	W ₂ 14	W ₂ 7	C10	W ₂ 12
	Gen. 3	W6	W8
<i>Internodes</i>										
18	1†
19	1
20	1
21
22	1
23	3
24	2
25
26	1†	1	1
27	1	1	3	2
28	4	1	3	1	3	1	1‡
29	1	1	1	2	2	1‡
30	2	1
31	1
32
33
34
35	2†

^a See note a to table 5.

TABLE 23

Cultures of 1912. Same as table 22, for doubles.^a

Ancestry	Gen. 1	WS1	WG9	WL10		WS1	WL10		WG9	WS1
	Gen. 2	W ₂ 16	C10	W ₂ 2	W ₂ 3	W ₂ 1	W ₂ 14	W ₂ 7	C10	W ₂ 12
	Gen. 3	W6	W8
<i>Internodes</i>										
12	1A
13
14
15
16
17	1
18	1
19	1
20	1	1
21	2	1	2
22	1	2	1	2
23	1	1†	1
24	1	3	1	1
25	5	3	2	3	2	1	1
26	1	2	3	1	3	2	1
27	2
28	3†	1†
29
30	1
31	1

^a See note a to table 5.

ably due to some cultural accident, perhaps to an excess of moisture in this row of pots.

The lots of plants may seem rather absurdly small for their purpose, but the uniformity of development here, with the marked normal divergence in internodes of the types in question, seems to justify a fair degree of confidence. Ten plants here were probably worth fifty in the field.

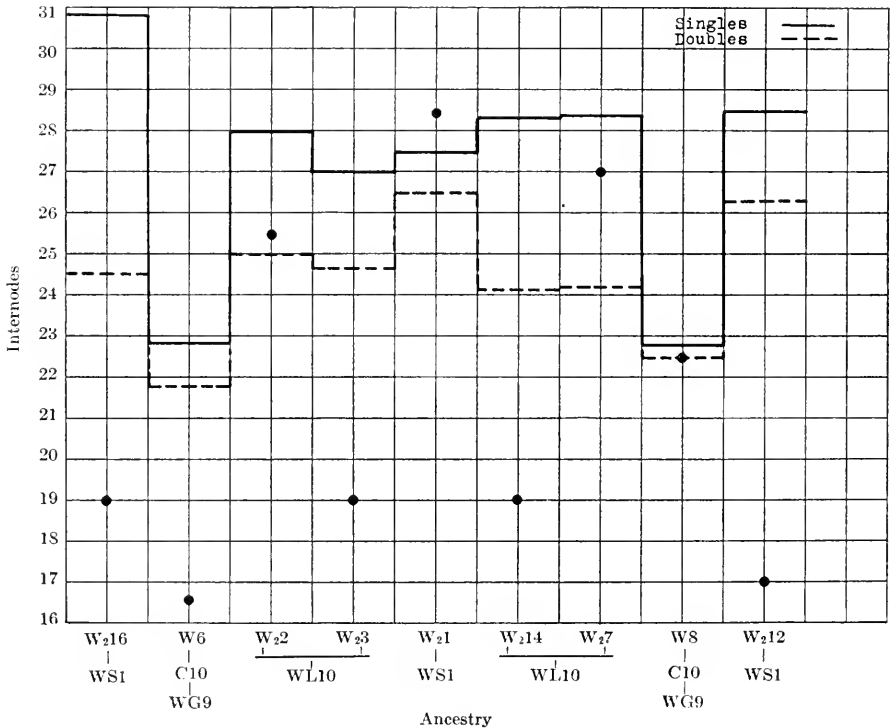


Chart 4. Cultures of 1912. Internodes: parental values and progeny means, shown as in chart 1. The true parental values are twice those indicated by the ordinate figures, which apply directly to the progeny values.

This test, with that of 1910, shows very positively that WS1-W₂16 was only phenotypically few-noded. Evidently WG9-C10-W8, the parent of field lot 22, really carried the earliness factor, as was somewhat doubtfully inferred from the field results; the five progeny of WS1-W₂12, on the other hand, though from a fewer-noded parent, have values that make the presence of the earliness factor improbable.

On the main point at issue the evidence seems satisfactory. Neither of the two very early and few-noded progeny of WL10 represented

shows in its progeny any evidence of belonging to the early type; the means are slightly lower than for the many-noded sibs of these parents, but far less so than with the parents descended from WG9-C10.

We conclude, then, that WG9-C10 was probably a monohybrid, and that the early-bearing gamete entering into its composition was of unknown but presumably mutative origin.

Most of the extracted late or many-noded parents may now be selected with practical certainty. WG9-C10-C8 and C1 (lots 5 and 6 in the 1911F cultures) and WG9-C10-M7 and M8 (lots 13 and 14) were genetically very similar to the check parents, as has already been concluded for two of them from the greenhouse cultures; presumably they were pure Snowflake.

The data for WG9-C10 itself (lot 26) seem to indicate that the results from the last eight lots are of very doubtful value; still, they show, especially in the original individual records, some evidence of the earliness factor which must be present in part of the individuals. The poor and slow germination of the old seed available may have had an important influence on the result; many of the early embryos may have been non-viable, and the seedlings may have been weaker than those from fresh seed. The 1911 data and observation of the plants in the field suggest that WG9-C10-W7, W3, and W10 (lots 23, 24, and 25) are the only remaining extracted late parents, WG9-C10-W5 and W8 (lots 21 and 22) carrying the earliness factor, as the four parents just preceding them in the cultures obviously did. Tables 22 and 23 confirm this conclusion for WG9-C10-W8.

It is presumably impossible to make a positive separation of the parents homozygous for the presence of the early factor. The greenhouse data suggest that WG9-C10-M4 was a pure early individual; the field data (see lot 9) agree, and suggest that WG9-C10-M9 (lot 10) and perhaps WG9-C10-M6 (lot 11) belong in the same class. WG9-C10-C2, C5, and C10 (lots 3, 4, and 40)¹² were all evidently heterozygous. Of the parents grown in house W, it would seem that only WG9-C10-W11 (field lot 19) was homozygous early. We have, provisionally, for the available single progeny of WG9-C10:

	House C	House M	House W	Total
Pure early	0	3	1	4
Hybrid early.....	3	1	5	9
Pure late	2	2	3	7
				20

¹² Statistical data given for the last only for the 1910 cultures, not for this field lot.

This corresponds well enough with the monohybrid expectation of 5:10:5; in fact, the deviation is just such as would be expected if there was occasional cross pollination of the unprotected flowers of WG9-C10 from Snowflake plants. The large proportion of evidently pure late parents is strong evidence for the monohybrid nature of WG9-C10.

The proportions of the two types among the doubles can only be estimated. The 1908 data suggest that 5 of the 10 doubles there reported were early; this number, with the 13 singles so classed, makes a total of 18 early-type plants out of 30. The ratio is slightly nearer to 1:1 than to 3:1, and the former proportion would suggest the peculiar type of inheritance found with the mutant types yet to be described. The evidence of the 1910 distributions, however, shows that the early type largely predominates in the next generation with both singles and doubles, and apparently this is true even when we exclude the progeny of the one parent classed as pure early.

The early factor can be positively detected only by progeny tests. No test has shown the presence of this factor elsewhere than in WG9-C10 and part of its descendants. WG9-C10 produced the early and Snowflake types among 20 single progeny nearly in the typical monohybrid proportions. Inspection of the double progeny in two generations suggests similar or possibly somewhat lower proportions there. A vicinistic origin for WG9-C10 is improbable. Presumably, then, the early type arose from Snowflake by a single factor mutation, the dominant mutant factor being inherited without special complications. We shall now consider certain apparently mutant types which are characterized by peculiar genetic behavior.

2. THE SMOOTH-LEAVED TYPE

This type was first observed in the cultures of 1908 (table 1) and has occurred frequently in later cultures (table 3). It is perhaps the mutant type of most frequent occurrence among progeny of Snowflake or early parents; 2410 unselected progeny from house-sown seed of such parents (see table 28) included 28 apparent mutants (14 singles, 11 doubles, and 3 undetermined), a mutation coefficient of $1.16 \pm .15$ per cent.

As grown in the greenhouse at Ithaca, this type (fig. 7, tables 12 and 13) was often many-noded, with correspondingly late flowering. Its most striking peculiarity, shown especially by young seedlings and not evident in the figures, was a lack of buckling between the veins

TABLE 24
Smooth-leaved type: heredity of apparent mutants and their progeny.^a

Parents	Cultures	Seeds ^b	Total examined		Plants			Other types			
			Undetermined	Determined	Smooth-leaved		All		Snowflake		
					Single	Double			Single	Double	All
26a	1911F	123	0	65	18	44 (46)	(1)
26b	1910A ^c 1911F 1910A ^c 1911F	79	0	29	1	2	6	6	5	22	1
26c	All P ₁ smooth 26b-10 26c-6	42, 15	3	21	2	4	8	2	3	11 (13)	0
26c-8	All P ₁ smooth 1911H ^c 1913, 1914 1911H ^c 1913, 1914	244, 217	3	115	3	6	32	8	8	77 (81)	1 (2)
All F ₁ smooth	All	118	0	8	1	0	1	2	4	6	1 (1)
All F ₁ smooth	All	118	2	56	0	2	11	20 (22)	19 (20)	40 (43)	2
All	All	118	3	55	6	6	19 (20)	12 (13)	16 (18)	28 (31)	1 (4)
All	All	256	5	119	7	8	31 (32)	34 (37)	39 (42)	74 (80)	3 (7)
All smooth	All	500, 217	8	234	10	14	63 (64)	42 (45)	47 (50)	151 (161)	4 (9)
26b-4	1911H	20	0	19	0	6	11 (13)	17 (19)	0
26c-3	1913	50	0	41	0	24 (25)	13 (15)	37 (40)	1
26c-8-8	1913	95, 50	2	73	0	33 (34)	38	71 (72)	1
26c-8-15	1913	23	0	20	0	11 (12)	7	18 (19)	1
26c-14	1911H	20	0	20	0	7 (8)	10	18 (19)	(1)
All	All	208, 50	2	173	0	81 (85)	79 (83)	161 (169)	3 (4)

^a For an explanation of the plan of this and the following tables, see the statement commencing the discussion of this table.

^b In each case the first number of seeds is the total planted. The numbers from unguarded flowers are given in italics, and those from doubly guarded flowers are marked with an asterisk.

of the leaves, and of general convexity of the upper surface of the leaves. Mature plants developed under favorable conditions in the greenhouse closely resembled Snowflake; the leaves, however, were noticeably brittle, and the dry capsules so brittle that it was often necessary, as it was not with Snowflake, to shell the seeds individually. Probably the fibrovascular system is in some way defective; *Oenothera rubrinervis*, which is also brittle (de Vries, 1906, lecture 18), has thin-walled bast fibers.

In the field cultures, both at Ithaca (fig. 5) and at Riverside, under conditions less favorable on the whole to the initiation of flowering, this type (fig. 8) differed much more widely from Snowflake. Flowering was excessively delayed, and the plants often remained low, with few branches, and rosette-like, with thin, rather narrow leaves. Small brown dead spots, possibly due to excessive transpiration, occurred so frequently on the leaves as to constitute a good diagnostic character for the type. Another peculiarity observed in the field is a reflexed position of the tip of the young leaf when first visible—Snowflake leaves being completely erect from the first.

In the 1914 cultures, with better development than in other field cultures, some smooth-leaved plants (figs. 9 and 10) were again more like Snowflake, though later and evidently more leafy.

Six smooth-leaved parents have been used in progeny tests, three of these being apparent mutants and three being F_1 progeny of two of those mutants. The results are presented in tables 24 and 25; these tables require a brief explanation, which will apply also to the similar tables for other types.

For the plan of the new pedigree numbers here used, see "Methods." The initial plants of a series are designated as the P_1 generation in the tables, their progeny as F_1 , etc. In table 24 the cultures are arranged according to their generations and their pedigree numbers under each generation; the smooth-leaved parents (P_1 or of the P_1 type) are given first, followed by the extracted Snowflake parents. In table 25 "good germination" indicates that in all lots included (taken as grown, not as summed by parents in table 24) the number of plants determined exceeds 50 per cent of the number of seeds sown, and *vice versa*; the weighted mean percentages obtained by dividing the total numbers of plants by the respective total numbers of seeds are given for each table in a footnote.

All six smooth-leaved parents (tables 24 and 25) gave mixed progeny, part smooth-leaved and part Snowflake. The surprising

fact is that the parental (smooth-leaved) type appears not in three-fourths of the progeny, but in only about one-fourth.

The extracted Snowflake parents tested behave like pure recessives, showing no influence of their smooth-leaved ancestry. Only the aberrant ratio seems inconsistent with the assumption that the smooth-leaved individuals tested were ordinary heterozygous dominants.

The relatively weak growth of this type and the apparently poor germination of the seed produced by it suggest that normal segregation may be masked by selective elimination. Possibly the smooth-leaved

TABLE 25
Smooth-leaved type: heredity. Summary.

Parents	Progeny					
	Cultures	Seeds	Plants			
			Total examined		Smooth-leaved	
			Undetermined	Determined	Number	Per cent
All smooth-leaved	Ithaca	304, 217	7	156	40	25.6 ± 2.4
All smooth-leaved	Riverside	196	1	78	23 (24)	30.8 ± 3.4
All smooth-leaved (6)	All	500, 217	8	234	63 (64)	27.4 ± 2.0
All P ₁ smooth-leaved (3)	All	244, 217	3	115	32	27.8 ± 2.8
All F ₁ smooth-leaved (3)	All	256	5	119	31 (32)	26.9 ± 2.8
All smooth-leaved	Germination good	293, 138	8	187 ^a	55 (56)	29.9 ± 2.2
All smooth-leaved	Germination poor	207, 79	0	47 ^a	8	17.0 ± 4.4
All Snowflake (5, F ₁ and F ₂)	All	208, 50	2	173	0	0

^a Respectively 63.8 and 22.7 per cent of the numbers of seeds planted.

factor is lethal when homozygous, as is often the case (Muller, 1918) with dominant mutant factors in *Drosophila*; the data for germination, however, indicate that two-thirds of the mature embryos can hardly belong to the mutant type. We might expect, in view of the weak growth of smooth-leaved plants, that partial elimination of heterozygotes would also occur. That this is the case is suggested, though the numbers are small, by the lower proportion of the mutant type with poor germination (table 25; see also tables 39 and 40); it should be noted, however, that transferring the first lot of table 24, the only lot between 50 and 73 per cent, to the "poor" total, makes the percentages practically identical.¹³

¹³ See also table 2 and the second paragraph under "Occurrence of Mutants."

In connection with the question of lethal action we must consider the inheritance of doubleness of flowers. Snowflake seed regularly gives a mixture of singles and doubles, about 53 per cent being doubles. The doubles, which are totally sterile, are probably (Frost, 1915) pure recessives (dd) for a single-double factor pair. The singles are always heterozygous (Dd); crosses with pure single races (Saunders, 1911) show that the approximately 1:1 ratio and the failure to produce pure singles, with self pollination, are due to the fact that all the functional pollen is doubleness-carrying (d). The excess of doubles over 50 per cent has been explained by Miss Saunders (1911) as due to heterozygosis of the singles for two linked complementary factors necessary to singleness, and by the present writer (Frost, 1915) as due to lower viability of the "single" gametes or embryos. The absence of functional single-carrying pollen is apparently due to a lethal factor acting after separation of the microspore tetrads, since the tetrads themselves appear normal.

In any consideration of factors linked with the single-double pair, this semisterility of the pollen must be remembered. For example, any dominant factor completely coupled with D in pollen formation would be totally absent from the functional pollen, and the zygotes produced by selfing would show directly the strength of linkage in the ovules.

The available data for the smooth-leaved type (table 24) are far from constituting an adequate test of linkage, but they suggest that the factors are independent. Certainly no high degree of linkage is indicated by the totals, nor do the detailed data suggest that smooth-leavedness is coupled with singleness in some parents and with doubleness in others.

We must admit that the peculiar inheritance of this type is not yet positively explained. Evidently larger cultures are needed, and crossing with the Snowflake type and with other commercial varieties; cytological study may also be required. Certain comparisons and speculative possibilities deserve mention, however, especially since the types yet to be discussed furnish additional evidence bearing on them. We may compare the smooth-leaved and double types, as follows:

DOUBLE	SMOOTH-LEAVED
1. A rare mutation of pure single ("normal").	1. Apparently a common mutation of pure Snowflake ("normal").
2. Recessive; extracted recessives are sterile mutant-type plants.	2. Apparently dominant; extracted recessives are fertile normal plants.

DOUBLE

3. Homozygous dominants not produced by hybrids, because functional pollen carries recessive factor only.
4. Recessive (mutant) type the more vigorous.
5. Dominant factor or another factor very closely linked with it is incompatible with formation of functional pollen.
6. Recessive type exceeds the expected equality by about 3 per cent among some 7000 individuals.

SMOOTH-LEAVED

3. Homozygous dominants perhaps not produced by hybrids.¹⁴
4. Recessive (normal) type the more vigorous; difference much greater than with single and double.
5. Relation of dominant factor to viability of pollen not yet determined.
6. Recessive type exceeds equality by about 23 per cent among 234 individuals.

The most probable hypothesis for smooth-leavedness, then, would so far seem to be essentially the same as for doubleness—complete elimination of the weaker type in pollen formation, and partial elimination in embryo-sac formation. Reciprocal crosses with Snowflake are obviously necessary; as we shall soon see, three of the other mutant types *have already proved to be carried by both eggs and sperms*.

The case of *Oenothera lata* (Gates, 1915) suggests the possibility that the smooth-leaved form might arise by reduplication of a chromosome. With ordinary *O. lata* the pollen is sterile, but pollination by *O. lamarckiana* gives about 15–20 per cent of *lata*. This deficiency of *lata* individuals is due, it seems, to a frequent loss of the extra chromosome at meiosis in *lata* ovules, with a resulting formation of more than 50 per cent of seven-chromosome (*lamarckiana*) eggs.

If the smooth-leaved type originates through duplication of a chromosome, we might suppose that other types of similar heredity involve other pairs of chromosomes. The apparent parallel with *O. lata*, which Bartlett (1917) has noted, was long ago suggested by the data, but with at least two or three types to be described linkage phenomena have seemed to conflict with this interpretation. Possibly different processes have produced different mutant types as with *Oenothera*; as we have considered types suggestive of *O. rubrinervis* (early) and of *O. lata* (smooth-leaved), we may consider next a form which in appearance is remarkably suggestive of *O. gigas*.

¹⁴ This possibility is only suggested by these cultures, but it becomes highly probable when the data for other types are considered.

TABLE 26^a
Large-leaved type: heredity of an apparent mutant and its descendants

Parents	Cultures	Seeds	Progeny						Other types	
			Total examined		Plants			Snowflake		
			Undeter- mined	Deter- mined	Large-leaved	All	Single	Double		All
28a	1913, 1914, & 1915-16	122	73	23	14 (16)	38 (40)	13 (14)	12 (17)	25 (31)	2
28a-1	1911	24	6	0	2 (3)	2 (3)	0	0 (3)	0 (3)	0
28a-8	1911	48	17	2 (3)	3 (4)	5 (7)	3 (5)	5	8 (10)	0
28a-9	1914	48	17	1 (2)	5 (6)	7 (9)	2	2 (6)	4 (8)	0
28a-20	1915-16	24	20	7	2	12	1	7	8	0
28a-37	1915-16	24	16	1	1 (3)	2 (5)	5	6	11	0
28a-46	1915-16	24	17	1 (2)	6 (7)	8 (10)	2 (4)	3	5 (7)	0
28a-47	1915-16	24	13	2	7	9	0	4	4	0
28a-53	1915-16	24	19	2	3	6	7	6	13	0
28a-55	1915-16	24, 7*	14	3	3	6	2	6	8	0
28a-57	1915-16	21, 11	18	1 (2)	1 (4)	2 (6)	2	6 (8)	8 (11)	1 ^b
28a-62	1915-16	24	17	5 (6)	4	9 (10)	1 (2)	4	5 (6)	1
28a-66	1915-16	24	18	5 (6)	5 (6)	10 (11)	4	3	7	0
28a-67	1915-16	24	20	1 (2)	5 (6)	7 (9)	6	4	10	1
28a-71	1915-16	24, 19	11	2	0	3	5	2	7	1
28a-73	1915-16	24*	7	2	0 (1)	2 (3)	2 (3)	1	3 (4)	0
28a-F ₁	All	408, 31*, 342	230	35 (42)	47 (58)	90 (109)	42 (48)	59 (68)	101 (117)	4
28a-8-30	1915-16	24	18	3	3 (4)	8 (9)	5	2 (4)	7 (9)	0
28a-8-38	1915-16	24	11	3	3	6	3 (4)	1	4 (5)	0
28a-9-2	1915-16	48	6	0	2	2	3	1	4	0
28a-9-28	1915-16	24	19	6	3	9	4	5 (6)	9 (10)	0
28a-F ₂	1915-16	90	54	12	11 (12)	25 (26)	15 (16)	9 (12)	21 (28)	0
All large- leaved	All	620, 31*, 432	357	70 (77)	72 (86)	153 (175)	70 (78)	80 (97)	159 (176)	6
28a-75 (Snowflake)	1915-16	21*	15	0	0	0	7	8	15	0

^a See notes to table 24.

^b Appearing like large-leaved slender.

It should, however, first be noted that, as will appear later, phenomena of apparent linkage in the case of certain other types (crenate, slender, and narrow) suggest that these forms commonly arise from Snowflake by segregation rather than by immediate mutation. The obvious objection to this hypothesis is the fact that the apparently mutant types seem to be dominant to the "normal" or Snowflake type. This objection can be met by assuming the presence of dominant inhibiting factors in the Snowflake parents that give apparent mutants.¹⁵

If the apparent mutants of the smooth-leaved type are thus produced by crossing over in a set of balanced factors, the lethal "balancing" the smooth-leaved factor itself may be distinct from that which sterilizes the singleness-carrying pollen. In considering the results here reported, therefore, we must always bear in mind the possible presence of several unidentified lethal factors. If the apparent absence of linkage between the smooth and double factors is not misleading, we must suppose that these factors are carried by different pairs of chromosomes; considerations advanced by Muller (1918, pp. 479-482), however, make it rather probable that the commoner types of apparent mutants here discussed are all due to factors carried by one pair of chromosomes, the pair containing the factor for doubleness and its normal allelomorph.

3. THE LARGE-LEAVED TYPE

A double of this type probably occurred in the 1907 cultures, though its appearance attracted so little attention that no record was made. In the field cultures of 1911 (table 3) several individuals suggested a *gigas* type, though there seemed to be intergradation with Snowflake. In the 1912 cultures a single with leaves "long, rather narrow, thick" developed normally and produced an abundance of good seed; from this individual (28a) all cultures of this type are descended.

This type is stout and coarse throughout, and late to flower. The leaves are strikingly long, thick, and rigid, though as a rule relatively

¹⁵ A letter suggesting this explanation was received from Dr. Muller soon after the same idea had been outlined in the "General Discussion" section below. Dr. Muller kindly gave further attention to difficulties at first encountered by the present writer, materially assisting in the formulation of an apparently tenable form of the hypothesis. Since, however, this scheme may seem "far-fetched" and unduly complex, it appears desirable to leave the original discussion of the individual types substantially unchanged. When the difficulties encountered by the assumption of frequent true mutation have been more fully presented, the need for some such addition to the scheme will be more evident.

narrow; under unfavorable weather conditions the flowers are often few and defective, while the leaves are resistant and long-lived (fig. 11). Figures 12 and 13 show well the coarse leaves and lateness of well developed large-leaved plants in the 1915-16 cultures, the plants in the latter figure being several weeks the older.

The results of the progeny tests are given in tables 26 and 27. All the twenty large-leaved individuals tested have given mixed progeny; the proportion of the mutant type, though much larger than with

TABLE 27
Large-leaved type: heredity. Summary.

Parents	Progeny					
	Cultures	Seeds ^a	Plants			
			Total examined		Large-leaved	
			Undetermined	Determined	Number	Per cent
28a	1913, 1914, & 1915-16	122	2	73	38 (40)	54.8 ± 3.9
28a-F ₁ (3)	1914	120	2	40	14 (19)	47.5 ± 5.3
28a-F ₁ (12)	1915-16	288	2	190	76 (90)	47.4 ± 2.4
28a-F ₂ (4)	1915-16	90	0	54	25 (26)	48.1 ± 4.6
28a-F ₁ & F ₂ (19)	All	498	4	284	115 (135)	47.5 ± 2.0
All large-leaved (20)	All	620	6	357	153 (175)	49.0 ± 1.8
Large-leaved	Germination good	360	3	260 ^b	115 (131)	50.4 ± 2.1
Large-leaved	Germination poor	260	3	97 ^b	38 (44)	45.4 ± 3.4
Snowflake (1, F ₁)	1915-16	24	0	15	0	0

^a Mainly from unguarded flowers; see table 26.

^b Respectively 72.2 and 37.3 per cent of the numbers of seeds planted.

smooth-leaved, approximates to 50 per cent, not 75 per cent, with little indication of selective elimination with poor germination.¹⁶

Here plainly, as with smooth-leaved, no pure mutant-type parent has yet been tested. Since this is also true of the other types, aside from early, that have been somewhat extensively tested, and fifty-three mutant-type parents in all have given Snowflake progeny, it is probable that homozygous individuals of these types seldom or never develop. The actual adult ratio with large-leaved is plainly not 2:1, but rather 1:1, a fact that would suggest absence of the mutant-type factor or factors from the pollen. The small trial cultures started in 1917, however, show that the type is carried by both sperm and eggs.

¹⁶ Since hybrids are of the mutant type in appearance, the possible cross pollination by Snowflake parents could hardly give Snowflake progeny with any pure large-leaved parent. It may, however, have reduced slightly the proportion of large-leaved progeny from heterozygous parents of this type.

If we are dealing here with a type cytologically like *Oenothera gigas*, or rather the triploid *semigigas*, abnormal distributions of chromosomes may occur at meiosis, giving unpredictable genetic results. There has been special difficulty, as the numbers of doubtful individuals in table 26 suggest, in separating large-leaved from Snowflake, though in part of the cases the difference is extreme. Possibly some of the doubtful individuals are genetic intermediates due to irregular meiosis in triploid nuclei; such irregularities in division (Gates, 1915) occur with *Oenothera*. Both cytological examination and crosses with Snowflake are plainly required.

TABLE 28

Crenate-leaved type: numbers of apparent mutants and association of the type with singleness of flowers.

Culture	Progeny of Snowflake and early parents				
	Total examined ^a	Crenate-leaved			Coefficient of mutation
		Single	Double	All	
1908	725 ^b	6	1	7	.97 ± .22
1910	338	3	0	3	.89 ± .32
1911F, seed house-sown	2072	13	3	16	.77 ± .13
All above	3135	22	4	26	.83 ± .11
All unselected	2410	16	3	19	.79 ± .12

^a See note *b* to table 2.

^b See note *c* to table 1.

4. THE CRENATE-LEAVED TYPE

This type (tables 1 and 3) is one of the three aberrant types of most frequent occurrence in the cultures here described, having constituted (table 28) about .79 per cent of the progeny of Snowflake and early parents. A large majority of the individuals have been singles, as table 28 shows. If the apparent mutants are produced by some process of segregation of factors, evidently the crenate and single factors were usually coupled in this material; if they are produced by immediate factor mutation, or are individually due to some change in a particular locus, evidently that locus is linked with the single-double locus and the change is more frequent in the single-carrying chromosomes; and finally, if they are due to reduplication or loss of a chromosome, the apparent linkage remains to be explained.

The margins of Snowflake leaves vary from entire or slightly sinuate to coarsely and irregularly dentate or serrate, this characteristic being subject to much environmental modification and varying

TABLE 29^a
Crenate-leaved type: heredity of apparent mutants and their descendants.

Parents	Cultures	Seeds	Progeny										Other Types
			Total examined		Crenate-leaved			Snowflake			All		
			Undeter- mined	Deter- mined	Single	Double	All	Single	Double	All			
22a	1911F & 1912	135, 28*, 107	0	25	2	0	1	0	2	19 (20)	1		
22b	1913	49	2	39	7	2	9	4 (5)	21 (25)	28 (30)	0		
22c	1910 & 1911F	130, III	2	25	3	0	4	0	10	21	0		
22d	1910 & 1911F	115	0	42	4	1	9	2	7	30 (31)	1 (2)		
All P ₁ crenate	All	420, 28*, 278	4	131	16	3	26	6 (7)	43 (44)	98 (102)	2 (3)		
22a-1	1913 & 1914	74	0	30	6	2	8 (9)	1 (2)	18	19 (20)	1		
22a-5	1913	50	0	39	4	1	6	5	26	31	2		
22c-1	1911H	20	0	18	3	2	5	3	9	12	1		
22c-7	1911H	20	1	17	4	1	5	2	7	9	2 (3) ^b		
22c-13	1911H	20	0	16	4	1 (2)	5 (6)	0	9	9	2 (1)		
22d-8	1911H	20	0	18	3	1	4 (5)	3	8	11	2		
22d-9	1911H	20	0	19	5	4	9	5	5	10	0		
22d-12	1911H, 1913, 1914	168	4	109	20	13 (14)	33 (35)	8	57 (62)	65 (70)	4		
22d-15	1911H, 1913, 1914	168	3	108	39	8	48	8	44 (46)	52 (54)	3 (6) ^c		
All F ₁ crenate	All	560	8	374	88	33 (35)	123 (128)	35 (36)	183 (190)	218 (226)	15 (20)		

TABLE 29^a—(Continued)
Crenate-leaved type: heredity of apparent mutants and their descendants.

Parents	Progeny										
	Cultures	Seeds	Total examined		Crenate-leaved			Snowflake			Other types
			Undeter- mined	Deter- mined	Single	Double	All	Single	Double	All	
22c-7-7	1913	54	1	37	5	5	10	4	19 (21)	23 (25)	1 (2)
22d-12-8	1915-16	22	0	17	2	2	4	3	10	13	0
22d-15-13	1913	100	3	58	14	6	21	5	27 (28)	32 (33)	2 (4)
22d-15-28	1914	10	0	1	0	0	1	1	0
22d-15-32	1914	28	0	2	0	0	1 (2)	1 (2)	0
22d-15-66	1915-16	8	0	7	0	1	6	7	0
22d-15-98	1915-16	6	0	6	0	1	5	6	0
All F ₂											
crenate	All	228	4	128	21	13	35	14	69 (73)	83 (87)	3 (6)
All crenate	All	1217									
		28*, 218	16	633	125	49 (51)	184 (189)	55 (57)	295 (307)	399 (415)	20 (29)
22b-39	1914	48	0	4	0	(1)	1 (3)	1 (4)	0
22d-2	1911H	20	0	19	0	5	13	18	(1)
All Snow- flake (2, F ₁)	All	68	0	23	0	5 (6)	14 (16)	19 (22)	(1)

^a See notes to table 24.

^b The doubtful plant was suggestive of a combination of the crenate and narrow types.

^c One of these doubtful plants perhaps crenate.

with the position of the leaves on the plant. In the erenate-leaved type this character is much accentuated, as can be seen by comparing figure 14 with figures 1 and 3; a warm greenhouse (fig. 14, upper line) gave very marked serration, while a cool greenhouse (lower plant, and also fig. 15) produced leaves much more nearly entire.

Under the much more extreme conditions of insolation, temperature, and humidity at Riverside, this type was often much dwarfed in comparison with Snowflake (figs. 16 and 17; see also fig. 23). In general, growth is weaker than with Snowflake and the stems more slender. Buds and flowers are often produced in great abundance, but the capsules are relatively few, small, and few-seeded. See tables 12 and 13 for internode data.

The progeny tests (table 29) show a slightly higher proportion of mutant-type progeny than occurred with smooth-leaved. A striking new feature appears for the first time in these results, the regular presence of linkage, or an association simulating linkage, with the single-double allelomorphs. Further, in all the four apparent mutants tested the erenate factor seems to be coupled with singleness, while among the sixteen F_1 and F_2 erenate parents there seem to be no crossovers.¹⁷ We seem to be justified, for reasons just given, in summing the progeny as in the tables. Two things appear at once in table 29: (1) there is a great excess of total doubles over the usual 53 per cent; (2) there is a much greater excess of doubles with Snowflake than of singles with erenate; (3) the supposed double-recessive class (Snowflake double) is about two and one-half times as large as the double-dominant class (erenate single).

Table 30 adds two features of special interest. First, there is good evidence of selective elimination with poor germination; compare the remaining percentages with those for "Ithaca, field," "1915," "P₁," and "Germination poor," and see tables 39 and 40; the only exceptional case is the low percentage for the thirty plants of 1915-16. It would be surprising if the slow and weak growth of the erenate plants did not lead to such a result. Second, there is evidence that the erenate individuals are smaller than Snowflake even before germination. The seeds of erenate parents are less uniform in size than those of Snowflake parents; small seeds are numerous, and even the larger ones probably weigh decidedly less than normal Snowflake seeds. With five erenate parents included in the cultures of 1913, random

¹⁷ With four of the parents the tests are obviously entirely inadequate; one other, 22d-9, gives no indication of linkage among nineteen progeny.

TABLE 30^a
Crenate-leaved type: heredity. Summary.

Parents	Cultures	Seeds	Progeny		Plants	
			Total examined		Number	Crenate Per cent
			Undetermined	Determined		
All crenate (10)	Ithaca, greenhouse	177, ⁷	4	158	54 (56)	35.4 ± 2.5
All crenate (3)	Ithaca, field	343, <i>211</i>	0	59	7	11.9 ± 4.0
All crenate (10)	Ithaca, all	520, <i>218</i>	4	217	61 (63)	29.0 ± 2.1
All crenate (7)	1913	503	11	361	116 (118)	32.7 ± 1.6
All crenate (5)	1914	158	1	25	3 (4)	16.0 ± 6.2
All crenate (3)	1915-16	36	0	30	4	13.3 ± 5.6
All crenate (12)	Riverside, all	697	12	416	123 (126)	30.3 ± 1.5
All crenate (20)	All	1217, <i>28*</i> , <i>218</i>	16	633	184 (189)	29.9 ± 1.2
All P ₁ crenate (4)	All	429, <i>28*</i> , <i>218</i>	4	131	26	19.8 ± 2.7
All F ₂ crenate (9)	All	560	8	374	123 (128)	34.2 ± 1.6
All F ₂ crenate (7)	All	228	4	128	35	27.3 ± 2.7
All crenate	Germination good	501, <i>28*</i> , ⁷ <i>211</i>	15	549 ^b	174 (178)	32.4 ± 1.3
All crenate	Germination poor	716, ⁷	1	84 ^b	10 (11)	13.1 ± 3.4
All Snowflake (F ₁ , 2)	All	68	0	23	0	0

^a See notes to table 24.

^b Respectively 76.7 and 16.8 per cent of the numbers of seeds planted.

samples of seed were sorted, and the smaller and larger seeds planted separately.

Table 31 gives the data from this test. Here is practically conclusive evidence (see tables 39 and 40) that the smaller seeds much more often contain embryos of the crenate type.¹⁸ Since the embryo of a *Matthiola* seed occupies practically all the space within the seed coats, it is evident that even as embryos Snowflake plants exceed

TABLE 31
Cultures of 1913. Crenate-leaved type: proportions from smaller and larger seeds of crenate parents.

Parent	Seeds		Progeny				
	Size	Number	Total determined	Crenate-leaved		Snowflake	Other types
				Number	Per cent		
22a-1	Smaller	21	6	4 (5)	83.3 ± 12.9	0	1
22a-1	Larger	29	23	3	13.0 ± 6.6	19 (20)	0
22a-5	Smaller	17	11	4	36.4 ± 9.5	6	1
22a-5	Larger	33	28	2	7.1 ± 6.0	25	1
22b	Smaller	13	8	5	62.5 ± 11.2	3	0
22b	Larger	36	31	4	12.9 ± 5.7	25 (27)	0
22d-12	Smaller	30	24	17	70.8 ± 6.5	5	2
22d-12	Larger	70	57	11 (12)	21.1 ± 4.2	42 (44)	1
22d-15	Smaller	32	24	17	70.8 ± 6.5	3	2 (4)
22d-15	Larger	68	54	18	33.3 ± 4.3	34 (35)	1
All	Smaller	113	73 ^a	47 (48)	65.8 ± 3.7	17	6 (8)
All	Larger	236	193 ^a	38 (39)	20.2 ± 2.3	145 (151)	3
All	All	349	266	85 (87)	32.7 ± 1.9	162 (168)	9 (11)

^a Respectively 64.6 and 81.8 per cent of the numbers of seeds planted.

crenate plants in size. This fact, obviously, is further evidence in favor of the hypothesis of partial selective elimination of crenate heterozygotes during embryonic development.

It may be worth noting that the 73 plants from the smaller seeds include 6 (8) apparent mutants of other types (mutation coefficient 11.0 per cent), while the 193 plants from the larger seeds include only 3 apparent mutants (1.6 per cent).

Before we can profitably discuss these data further, we must consider the results from cross pollination (tables 32 and 33). The numbers, though small, make it very probable that both eggs and sperms carry the crenate factor. Further, it appears from series 20 that only a small portion of the sperms carry this factor, as we should expect from its apparent linkage with singleness. If homozygotes are non-viable, the combined crenate percentages of reciprocal crosses should

¹⁸ The poorer germination of the smaller seeds suggests that the disparity between the two lots of seeds in the proportion of crenate embryos was even greater than the cultures indicate.

TABLE 32.^a
Hybridization of the Snowflake and crecate-leaved types; reciprocal crosses, F₁ generation.

Parents ^b	Cultures	Seeds	Progeny										Other types
			Total examined		Crecate-leaved			Snowflake			All	Other types	
			Under- mined	Detec- mined	Single	Double	All	Single	Double	All			
20a	1913	43	4	25	1	0	1	7 (8)	14	21 (22)	1 (2)		
20bb	1913	76	1	64	4	(1)	4 (5)	22	32 (34)	54 (56)	3		
20cb	1913	4	0	4	0	1	3	4	0		
First three	1913	123	5	93	5	(1)	5 (6)	30 (31)	49 (51)	79 (82)	4 (5)		
20dc, ed, & 20de	1914	163	0	14	0	3 (4)	6 (10)	9 (14)	0		
20de	1915-16	18	0	14	0.	2	2	7	4	11	1		
20ff	1915-16	50	0	45	1	0	1	17 (18)	22 (23)	39 (42)	1 (2)		
20gf	1915-16	17	0	12	0	0	1	5	5	10 (11)	0		
20gg	1915-16	15	0	14	0	0	1	5	7 (8)	12 (13)	0		
20hd	1915-16	20	0	18	1	0	1	7	10	17	0		
Last five	1915-16	120	0	103	3	3	6	41 (42)	48 (50)	89 (94)	2 (3)		
All of series 20	All	406	5	210	8	3 (4)	11 (12)	74 (77)	103 (111)	177 (190)	6 (8)		
21aa	1913	32	1	18	2	0	2 (3)	2	12 (13)	14 (15)	0		
21bb	1914	34	0	1	0	0	1	1	0		
21dd	1915-16	9	0	6	0	1	5	6	0		
All of series 21	All	75	1	25	2	0	2 (3)	3	18 (19)	21 (22)	0		
Snowflake parents of hybrids (5)	1913 & 1914	271, 50	3	134	(1)	0	(1)	59 (63)	60 (64)	119 (127)	3		

^a See notes to table 24.^b Snowflake is the seed-parent type in series 20, and the pollen-parent type in series 21.

exceed the percentage from selfed parents; the expected high proportion with series 21, however, might well be realized with adequate numbers and good germination.

In spite of the small totals, it is very probable that linkage similar to that of the selfed cultures prevails with series 21. Where the crenate type is the pollen parent (series 20) linkage ratios are on our hypothesis impossible, since the eggs are all Snowflake and the sperms all double; the data, however, though statistically inconclusive, suggest that the excess of singles with crenate and of doubles with Snowflake is greatly reduced but not abolished.

TABLE 33

Hybridization of the Snowflake and crenate-leaved types. Summary.

Parents	Progeny					
	Cultures	Seeds	Plants			
			Total examined		Crenate	
			Undetermined	Determined	Number	Per cent
20aa, bb, & cb	1913	123	5	93	5 (6)	6.5 ± 1.6
20dc, ed, & ic	1914	163	0	14	0	0
20de, ff, gf, gg, & hd	1915-16	120	0	103	6	5.8 ± 1.5
All of series 20	All	406	5	210	11 (12)	5.7 ± 1.1
21aa, bb, & dd	All	75	1	25	2 (3)	12.0 ± 4.4
Snowflake parents of hybrids (5)	All	271, 50	3	134	(1)	.7 ± .5

If we may ignore the doubtful correlation just mentioned a fairly adequate complete hypothesis for the selfing ratio is possible. Assume (1) a gametic ratio¹⁹ of $5DC:1dC:1Dc:5dc$, or 16 $\frac{2}{3}$ per cent of crossing over; (2) non-viability of homozygous crenate (CC); (3) low viability of simplex crenate (Cc), eliminating an average of 60 per cent of this type; and (4) coupling of D and C in all parents tested. Evidence has already been presented for assumptions (1), (2), and (3), except as to the intensity of linkage, while (4), as will be seen, is not at all improbable.

Random fertilization under these conditions, excepting (3), would give $26DdCc$ (crenate single) + $10ddCc$ (crenate double) + $5Ddcc$ (Snowflake single) + $25ddcc$ (Snowflake double). The other two classes, $5DdCC$ and $1ddCC$, would be non-viable pure crenate. Adding assumption (3) gives the following comparison:

¹⁹ Representing the singleness and doubleness factors by D and d , and the crenate factor and its "normal" allelomorph by C and c .

	DdCe	ddCe	Ddec	ddec
Theoretical ratio ($n = 44.4$).....	10.4	4	5	25
Calculated for $n = 540$	126	49	61	304
Observed ($n = 540$) ²⁰	125	51	57	307

This fit surely cannot be criticised, whatever may be thought of the devices employed to obtain it! With cross pollination the agreement is fairly good in the case of series 20, which gives the only fairly reliable data. We are assuming $16\frac{2}{3}$ per cent of crossover dC sperms; elimination of .60 of $16\frac{2}{3}$ per cent, or 10 per cent of the total, gives $.06\frac{2}{3}/.90 = 7.4$ per cent expected crenate, as against 5.9 per cent observed. Series 21 is supposed to have 50 per cent of C eggs in the ratio $5DC:1dC$; elimination of .60 of this proportion, or 30 per cent of the total, would leave $.20/.70 = 28.6$ per cent, against 12.0 per cent in the very inadequate material observed. An adequate test of the hypothesis obviously requires large hybrid cultures, from vigorous seed sown under favorable conditions for germination.

A scarcity of crossover crenate singles follows from the hypothesis; they constitute only one twenty-sixth of the total number of viable crenate single progeny of crenate parents. No direct evidence indicating that the crenate and double factors are ever coupled in singles has yet been discovered.

If the supposed crenate mutants are due to immediate factor mutation, however, it seems strange that the same locus is changed more readily in a singleness chromosome than in one carrying the doubleness factor, in a ratio similar to the linkage ratio of later generations. If the apparent mutants are really segregates from a balanced-lethal combination, the observed original coupling of crenate with single might be an accident of sampling involved in the original choice of material; other initial parents might give the reverse coupling.

5. THE SLENDER TYPE

This type is comparatively rare as an apparent mutant from Snowflake or early; the 3135 plants reported in table 28 gave only 4 (6) mutants (2 singles and 4 doubles, 2 of the latter perhaps Snowflake), a mutation coefficient not over .19 per cent. This type seems to occur more frequently among progeny of crenate, a type similar in some

²⁰ Omitting 29 plants classed as neither crenate nor Snowflake, which as probably non-crenate should perhaps be added to Snowflake, and also 64 plants (13 crenate and 51 Snowflake) with flower data incomplete. Complete data for the total of 633 plants would plainly give a somewhat poorer fit, but this could be improved by assuming a slightly greater elimination of Cc zygotes.

TABLE 34^a
Slender type: heredity of apparent mutants and their descendants.

Parents	Cultures	Seeds	Progeny										Other types
			Total examined					Plants					
			Undetermined	Determined	Single	Double	All	Single	Double	All			
25a	1913	19	1	1	(1)	0	0	(1)	0	0	
25b	1910, 1911F, & 1913	173, 21	0	83	9 ^b (11)	8 (9)	8 (9)	17 (20)	1	58 (62)	59 (63)	0	
All P ₁ slender 25b-6	All 1911H, 1913, 1914	192, 40	1	84	9 (12)	8 (9)	17 (21)	1	58 (62)	59 (63)	0		
25b-9	1911H, 1914	94	0	56	10	4	14	2 (3)	32 (37)	35 (41)	1		
25b-11 ^c	1911H & 1913	20	0	20	0	1	1	0	17	17	(2)		
25b-79	1913	70	1	31	4	11 (15)	16 (21)	0	8	8	1 (2)		
All F ₁ slender 25b-6-8 ^c	All 1913	39	0	1	0	0	1	1	0		
25b-6-8-6	1915-16	223	1	108	14	16 (20)	31 (36)	2 (3)	58 (63)	61 (67)	2 (5)		
All slender All	All	42, 53, 510, 82	0	7	2	1	3	0	4	4	0		
“extreme” slender	All	112, 42	1	38	6	12 (16)	19 (24)	0	12	12	1 (2)		
“ordinary” slender	All	398, 40	1	205	34 (38)	15 (16)	50 (55)	4 (5)	132 (141)	137 (147)	1 (3)		

^a See notes to table 24.

^b One of these “apparently” single.

^c Described as “extreme” specimens of the slender type.

respects, and *vice versa*. Under favorable conditions this type may closely resemble Snowflake, but is decidedly more slender in stems, leaves, and pedicels. A characteristic drooping of flowers and branches is well shown by two plants in figure 18; the single is 25b of the tables. The progeny of 25b shown in figure 19 illustrate a variability of the "slender" characteristics which has suggested the presence of genetic differences among plants classed as slender. The leaves often resemble those of crenate more closely than do Snowflake leaves.

In the field at Ithaca flowering was markedly earlier than with Snowflake, and the type seems to be earlier on the whole. The Riverside conditions have commonly given a decided dwarfing as compared with Snowflake, though not to the extreme degree that this has occurred with crenate (figs. 20 and 21).

The results of selfing tests are reported in tables 34 and 35. The distributions have the same general characteristics as with crenate, with some remarkable differences. The excess of doubles with Snowflake is very much greater, the ratio being about 30:1; with slender, however, the excess of singles is slight in the grand total and perhaps significantly variable with different parents.

Plant 25b-11, the "extreme" individual of figure 19, appears to give a real excess of slender over Snowflake, and of double slender over single slender, though the numbers are much too small for certainty. The two parents classed as "extreme" are (tables 39 and 40)²¹ quite probably genetically different from the other slender parents. It should be noted that plant 25b-6-8-6, progeny of one of the parents described as "extreme," has also given a relatively high proportion of slender progeny. Perhaps the "extreme" form is heterozygous for a second slenderness factor similar to the original one.

The percentages of mutant-type progeny are (table 39) much more variable than with smooth, large, or crenate, and (table 40) there is no good evidence of selective elimination; both these facts may depend on genetic differences among the parents tested.

The great modifiability of the various types, including Snowflake, indicated by a comparison of, for instance, figures 14, 15, and 16, greatly complicates the positive determination of types. In the cultures of 1911H and 1913, where crowing in flats or aphid injury in the field interfered with normal development of some plants, the impression was obtained that the slender type occurred in several grades

²¹ In the calculation of the probability of simple sampling, f is taken as 3 (the number of cultures), not 2 (the number of parents).

TABLE 35
Slender type: heredity. Summary.

Parents	Cultures	Seeds	Progeny			
			Total examined		Slender	
			Undetermined	Determined	Number	Per cent
All slender (4)	Ithaca, greenhouse	75	1	71	14 (18)	25.4 ± 3.7
All slender (1)	Ithaca, field	137	0	51	8	15.7 ± 4.4
All slender (4)	Ithaca, all	212	1	122	22 (26)	21.3 ± 2.9
All slender (5)	1913	182, 82	1	73	29 (34)	46.6 ± 3.7
All slender (2)	1914	63	0	4	0	0
All slender (1)	1915-16	53	0	44	18 (19)	43.2 ± 4.8
All slender (7)	Riverside, all	298, 82	1	121	47 (53)	43.8 ± 2.9
All slender (8)	All	510, 82	2	243	69 (79)	32.5 ± 2.0
All P ₁ slender (2)	All	192, 40	1	84	17 (21)	25.0 ± 3.4
All F ₁ slender (4)	All	223	1	108	31 (36)	33.3 ± 3.0
All F ₂ and F ₃ slender (2)	All	95, 42	0	51	21 (22)	43.1 ± 4.4
All slender	Germination good	199, 21	1	165 ^a	47 (55)	33.3 ± 2.5
All slender	Germination poor	311, 61	1	78 ^a	22 (24)	30.8 ± 3.6
"Extreme", slender (2)	All	112, 42	1	38	19 (24)	63.2 ± 5.1
"Ordinary", slender (6)	All	398, 40	1	205	50 (55)	26.8 ± 2.2

^a Respectively 82.9 and 25.1 per cent of the numbers of seeds planted.

probably unlike genetically. In the 1916 cultures, on the other hand, with better development, this type seemed substantially as uniform as the others.

If we ignore these possible genetic differences and attempt to apply the scheme worked out for crenate, difficulties appear at once. First, the scarcity of Snowflake singles would indicate much closer linkage than with crenate, while the relative abundance of slender doubles apparently contradicts this supposition. Second, the inadequate results from crossing with Snowflake (table 36) suggest that the sperms carry the supposedly crossover slender factor at least as often as do the eggs. While crenate as pollen parent gives results agreeing tolerably with the hypothesis, slender gives results differing from these in the wrong direction.

No doubt, however, the disagreements can be over emphasized. Both crenate and slender as seed parent seem to give the expected relations between singles and doubles, and series 23 also does this with the Snowflake progeny. Obviously the functional sperms and eggs of these mutant-type parents exhibit different ratios between types, and the peculiar results in other respects with slender may be related to the added complication suggested above. The astonishing feature of the data, of course, is the great excess of single slender over double slender in series 23—an excess which suggests an actual significant excess of singles in the totals of all types given by this cross—while with selfed slender there is a great total deficiency of singles. We may at least feel confident that the modifications of the single-double ratio, with this type and with crenate, are due to lethal action which also affects the proportions of viable slender and crenate gametes or zygotes.

If differential viability before germination is an important factor with these types, very probably it differs according as Snowflake or the mutant type is the seed parent, and according to the parental environment. In other words, partial selective elimination during seed formation may vary with the environment of the embryos, according as this environment is affected by either the genetic constitution or the external environment of the seed parent. Until such uncertainties are eliminated, we are hardly justified in ruling out, for the types discussed, the probability that regular segregation and (in the last two cases) true linkage are concerned in these phenomena. In fact, the definite differences in ratios between reciprocal crosses and between at least one of the crosses and selfing encourage further attempts at satisfactory factorial analysis.

TABLE 36
Hybridization of Snowflake and slender; reciprocal crosses, F₁ generation.

Parents ^a	Cultures	Seeds	Progeny										Other types
			Total examined			Slender			Snowflake			All	
			Undeter- mined	Deter- mined	Single	Double	Num- ber	All		Single	Double		
								Per cent of total					
23aa, bb, & cc	1914	114	1	6	0	0	1	1 (5)	2 (6)	0	
23ca	1915-16	25	0	23	4	1	5	21.7 ± 5.8	6	10	16	2	
23ca	1915-16	120	0	93	20	(1)	20 (21)	22.6 ± 2.9	32 (33)	33 (35)	65 (68) ^b	2 (4)	
All of series 23	All	259	1	122	24	1 (2)	25 (26)	21.3 ± 2.5	39 (40)	44 (50)	83 (90)	4 (6)	
24aa	1915-16	53	0	21	2	0	2	9.5 ± 4.3	0	18	18	1	
Series 23 and 24	Germination poor	167	1	27 ^c	2	0	2	7.4 ± 3.4	1	19 (23)	20 (24)	1	

^a Snowflake is seed parent in series 23, and pollen parent in series 24.

^b These three doubtful plants had leaves alone resembling slender, evidently because of ill health; one, recovering after pruning, lost its slender appearance. The doubtful slender double very probably also belongs here.

^c 16.2 per cent of the number of seeds planted.

6. THE NARROW-LEAVED TYPE

As table 37 indicates, this type competes with crenate for second place in frequency of occurrence in the Ithaca cultures; in fact, when only the strictly unselected cultures are considered the percentage is very close to that for smooth-leaved. A feature of special interest is the apparent association of the mutant type with doubleness.

In a cool greenhouse this type (fig. 22) varied from exceptionally late and many-noded to ordinary in both characters. The leaves (see also fig. 18) were typically narrow, rather strictly entire, often rolled backward or twisted, and typically more ascending than those of

TABLE 37

Narrow-leaved type. Numbers of apparent mutants and association of the type with doubleness of flowers.

Culture	Progeny of Snowflake and early parents				
	Total examined ^a	Narrow-leaved			Coefficient of mutation
		Single	Double	All	
1908	725 ^b	0	2	2	.28 ± .26
1910	338	1	4	6	1.78 ± .38
1911F, house-sown	2072	7	12	20	.97 ± .15
All above	3135	8	18	28	.89 ± .12
All unselected	2410	8	16	26	1.08 ± .14

^a See note *b* to table 2.

^b See note *c* to table 1.

Snowflake. The apex of the leaf is often more acute than with Snowflake, and many leaves are mucronate or at least end in a sharp, rigid tip.

A striking characteristic is the narrowness of the sepals, resulting in frequent early separation at the edges, partially exposing the petals in immature buds.

Under the less favorable field conditions the plants often remain long as dwarf rosettes, and flower late and feebly if at all. Figures 23 and 24 show comparatively well developed plants in the field.

The type is on the whole very distinct in the field, though there has been some question whether a greenhouse plant such as that in figure 18 is genetically different from those with short and rigid leaves (figs. 22 and 24); the very great variability in leaf form due to external conditions makes such a question very difficult without extensive progeny tests. It is now (1918) probable that narrow-dark (p. 143) was not distinguished from narrow in the greenhouse.

TABLE 38^a
Miscellaneous mutant types: heredity of apparent mutants.

Parents	Cultures	Seeds ^a	Progeny										
			Total examined		Parental type			Snowflake			Other types		
			Undetermined	Determined	Single	Double	Number	All	Single	Double	All	Single	Double
33a	1911H & 1913	41	0	22	0	1	1	8 (19)	8 (10)	16 (20)	1 ^c		
33b	1913	45	0	15	1	2	3	5 (6)	6	11 (12)	1		
All narrow-leaved	All	86, 45	0	37	1	3	4	13 (16)	14 (16)	27 (32)	2		
"Short stout" (1, P ₁)	1911H & 1913	39	0	29	0	3 (5) ^b	5 (7)?	10 (11)	11	21 (22)	0		
27a, "small convex-leaved?"	1913 & 1914	98	5	44	1?	1?	2 (4)?	9 (12)	16 (18)	25 (30)	3 (5)		

^a See note *b* to table 24.

^b One of these "evidently" double.

^c Narrow-dark-leaved type.

The few singles have produced few seeds, and these were highly variable in size. The capsule often has a defective septum, more or less of the distal portion being absent. Germination was poor in the small cultures secured (table 38, upper part), with only 10.8 per cent of the mutant type among the progeny.

This case agrees in most respects with those previously discussed, but adds one point of interest in the occurrence of apparent coupling of mutant type with doubleness rather than singleness. Seed appears to be less abundant and less well developed than with any of the preceding mutant types, facts probably significant in relation to the low percentage of narrow progeny from narrow parents, though the large probable error of the percentage must be considered.

7. MISCELLANEOUS ABERRANT TYPES

As part of the aberrant individuals occurring in the greenhouse were either doubles or singles that produced no seed, while practically no seed was produced by any plants in the field at Ithaca or by even some of the commoner mutant types at Riverside, the opportunity for progeny tests has been almost entirely limited to the types so far discussed.

The narrow-dark-leaved type (table 3) was common and distinct in the field at Ithaca, where it constituted about .48 per cent of the 2072 plants from house-sown seed, and has been readily identified in several cases at Riverside. It was not distinguished in the greenhouse cultures, but was very probably included under narrow-leaved. Possibly a single described as "small-convex-leaved" belonged to this type, though two field plants were given this name as distinct from narrow-dark; according to a photograph (fig. 25, second plant from left), another greenhouse plant (a double) may have been similar to narrow-dark-leaved. The narrow-dark-leaved type (figs. 26 and 27) has narrow dark-green leaves, strongly convex upward, and evidently tends to compactness of growth and lateness of flowering; under field conditions it seems decidedly more like Snowflake than like narrow-leaved.

The 44 progeny (table 38) secured from the greenhouse single mentioned above included 2 (4) narrow-dark-leaved individuals and 3 (5) other plants not Snowflake (the last including two smooth, one large, one slender, and one semicrenate), besides five undetermined plants. Plainly the type of the parent is still in doubt.

Another very different greenhouse plant, described as "stout dwarf" (fig. 25, third from left), gave among 29 progeny (table 38) 5 (7) individuals evidently not Snowflake, which may have been narrow-dark or may have belonged to another type that was somewhat similar under the conditions of the tests. The parent resembled Snowflake except in its short internodes and short, stout capsules.

Four other plants suspected of mutation apparently entirely failed to repeat their type in their progeny, perhaps because of the smallness of the house cultures. One of these was the plant, much branched for the warm greenhouse, third from the right in figure 18; another was a very late plant with a remarkably large number of main-stem leaves; the others were a plant with unusually small flowers and one with some of the leaves somewhat spatulate. Possibly all of these were Snowflake, though the second, which gave poor germination, probably was not. All these four plants have been included as Snowflake parents for tables showing numbers of apparent mutants.

The small-smooth-leaved type is well shown in figure 25 (first and fifth from the left). It is the smallest and weakest of the fairly common and definitely identified types; it has small, very smooth leaves, and is late in blooming. The two plants shown were both singles, but they set no seed.

The semicrenate-leaved type (table 3) differed slightly but apparently definitely from Snowflake, somewhat resembling crenate-leaved in leaf form. The one "pointed-crenate-leaved" plant of table 3 may have been crenate-leaved. The "compact" and "curly-leaved" plants of this table have not been identified with any aberrant types in other cultures. With the remaining six types of table 3 all the individuals have been questioned as possibly Snowflake; it is now practically certain that some of those in the second, third, and fourth groups belonged to the large-leaved type since studied, but the apparent intergradation with Snowflake makes any attempt at a definite reclassification from the records a matter of doubtful value.

The second plant from the right in figure 25 was remarkable for its short stem and few but large leaves. Several other more or less exceptional individuals have appeared in the cultures, especially among some plants with abnormal cotyledons, selected from large numbers of greenhouse seedlings in the 1908 cultures, which were examined for syncotyledony. Some of these were very weak plants which finally died without flowering.

The fluctuations in habit, leaf form, etc., within the type are such that the determination of familiar types is often a matter of some uncertainty, as is shown by data that have been presented. It may well be that among the doubtful types are included several definite but comparative rare mutant forms, which occurred too infrequently to afford adequate material for positive classification.

S. SOME PROBABILITIES OF RANDOM SAMPLING

For compactness of presentation and convenience of comparison the material in tables 39 and 40, to which some incidental references have already been made, is collected here rather than scattered through the discussions of the various types concerned. Some statements as to methods are also necessary in connection with each of the topics here treated.

First, it should be noted that the percentages previously given have regularly been accompanied by the probable errors of simple sampling. These probable errors have been calculated by the formula $E_{\text{per cent}} = .6744898 \sqrt{\frac{pq}{n}}$, where p is the percentage of the mutant type ("successes"), q is $1 - p$, and n is the size of the sample (the number of plants concerned).

In the heredity tables for each type, p has uniformly been taken as the percentage of the total of the lots compared, or p_0 .

For the "mutation coefficient" the percentage of the grand total of unselected house-sown lots has regularly been used. Evidently the few selected progeny included in tables 1, 28, and 37 should be omitted. All the percentages here are so low that the probable errors deserve little confidence, even though n is usually fairly large. The rather close agreement of the percentages of all apparent mutants in the three distinct lots of unselected house-sown cultures suggests that they represent fairly well the population value for the potentialities of the seeds; and even if the mean percentage of the total of the lots for the main comparisons is actually nearer, it is safer to use the larger probable errors resulting from the method here employed. Furthermore strict use of p_0 would sometimes require several slightly different probable errors for the same percentage, for use in different comparisons in the same table.

If the probable error of the difference of any two percentages in the same table is to be obtained, therefore, formulae corresponding to those given by Yule (1911, pp. 264-267) are applicable.

Now, it is possible in some of these cases to calculate the actual standard deviation of the percentage in subsamples which make up an aggregate sample. Table 39 gives such actual standard deviations, in comparison with the corresponding theoretical or expected standard deviations given by

$$\sigma_{\text{per cent}} = \sqrt{\frac{pq}{n-3}}$$

TABLE 39

Standard deviations of percentages of mutant types. Values derived from \sqrt{pq} , compared with values expressing the actual variability of subsamples.

Type of parent and grouping of progeny	N	f	\bar{n}	p	Standard deviation of samples of mean size n		
					Actual	Theoretical, $\sqrt{\frac{pq}{n-3}}$	Difference E σ
Smooth-leaved type:							
All lots by parentage	234	6	39.0	27.35	7.5	7.4 \pm 1.4	+ .1
All lots as grown	234	12	19.5	27.35	11.3	11.0 \pm 1.5	+ .2
Germination good	187	7	26.7	29.95	10.9	{ 9.4 \pm 1.7 9.2 ^a	+ .9
Germination poor	47	5	9.4	17.02	5.2	{ 14.9 \pm 3.2 17.6	- 3.0
Large-leaved type:							
All lots by parentage	357	20	17.85	49.02	10.7	13.0 \pm 1.4	- 1.6
All lots as grown	357	22	16.2	49.02	10.9	13.7 \pm 1.4	- 2.0
Germination good	260	14	18.6	50.38	11.3	{ 12.7 \pm 1.6 12.7	- .9
Germination poor	97	8	12.1	45.36	8.7	{ 16.5 \pm 2.8 16.6	- 2.8
Crenate-leaved type:							
All lots by parentage	633	20	31.65	29.86	10.6	8.6 \pm .9	+ 2.2
All lots as grown	633	28	22.6	29.86	12.5	10.3 \pm .9	+ 2.4
Germination good	549	20	27.45	32.42	10.7	{ 9.5 \pm 1.0 9.3	+ 1.2
Germination poor	84	8	10.5	13.10	10.5	{ 12.3 \pm 2.1 16.7	- .9
Seed-size test, smaller seeds	73	5	14.6	65.75	13.2	{ 13.9 \pm 3.0 13.8	- .2
Same, larger seeds	193	5	38.6	20.21	9.4	{ 6.7 \pm 1.4 7.9	+ 1.9
Same, all seeds, by parentage	266	5	53.2	32.71	10.3	6.6 \pm 1.4	+ 2.6
Same, all seeds, as grown	266	10	26.6	32.71	22.9	9.7 \pm 1.5	+ 8.8
Slender type:							
All lots by parentage	243	8	30.4	32.51	17.5	9.0 \pm 1.5	+ 5.7
All lots as grown	243	13	18.7	32.51	19.7	11.8 \pm 1.6	+ 4.9
Germination good	165	7	23.6	33.33	14.9	{ 10.4 \pm 1.9 10.3	+ 2.4
Germination poor	78	6	13.0	30.77	27.2	{ 14.6 \pm 2.8 14.8	+ 4.5
Parents "extreme"	38	3	12.7	63.16	14.4	{ 15.5 \pm 1.3 15.1	- .3
Parents "ordinary"	205	10	20.5	26.83	14.7	{ 10.6 \pm 1.6 11.2	+ 2.6
Narrow-leaved type:							
All lots as grown	37	3	12.3	10.81	8.1	10.2 \pm 2.8	- .75

^a The second values for some cases in this column are derived from p_n (see text).

For example, table 27 gives the percentage of large-leaved plants among the 357 progeny of the 20 large-leaved parents as 49.0 ± 1.8 per cent. This probable error is given by $.6744898 \sqrt{\frac{pq}{n}}$, where $p = 49.0$ per cent, $q = 51.0$ per cent, and $n = 357$. These 357 progeny, as table 39 indicates, came from 20 parents which contributed an average of 17.85 progeny each, and the actual standard deviation of the percentage in these 20 sibships was 10.7 per cent.

Obviously the expected standard deviation of simple sampling for comparison must represent samples not of 357 plants each but of 17.85 plants each. Now a percentage is obviously a mean (of values all either 0 or 1). Since "Student" (1908) has shown that the theoretical standard deviation of the mean in samples is given more exactly by

$$\sigma_{\text{mean}} = \frac{\sigma_{\text{variate}}}{\sqrt{n-3}} \text{ than by } \sigma_{\text{mean}} = \frac{\sigma_{\text{variate}}}{\sqrt{n}}$$

(the value for the normal curve conventionally used for the probable error of the mean) and since \bar{n} , the mean size of sample, is small enough to make the correction a matter of considerable importance, $\sqrt{\bar{n}-3}$ is here used. Since $\sigma_{\text{variate}} = \sqrt{pq}$, we have $\sigma_{\text{mean}} = \sqrt{\frac{pq}{\bar{n}-3}}$, where $\bar{n} = 17.85$. This gives a theoretical standard deviation of 13.0 per cent.²²

It is true (Yule, 1911, p. 260) that the ordinary method of calculation of the actual standard deviation is not satisfactory for means when the samples vary in size. A method has been used, however, which obviates this difficulty, so that comparison with the results given by $\sqrt{\frac{pq}{\bar{n}-3}}$ is strictly legitimate. Each squared percentage deviation has been weighted by multiplying it by the number of individual plants which it represents, and the summation of squared deviations has then been divided, not by Σf , the number of samples, but by $\Sigma f \times \bar{n}$, the number of samples multiplied by the mean weight or average size of sample (in other words, by N , the total number of individuals).²³

²² In the calculations for table 39 p has been taken as the percentage given in this table, to two decimal places, while with all other numbers employed in calculation, including $\bar{n}-3$, three or more decimal places have been used as needed.

²³ Algebraic proof of the correctness of the method has kindly been furnished by Frank L. Griffin, Professor of Mathematics, Reed College, Portland, Oregon. If it develops that this rather obvious device has not been suggested for the purpose, it is to be presented elsewhere with the mathematical proof. When the variates are not grouped in classes the calculation is substantially as easy as without weighting, while the theoretical value is found with much less work than by the method given by Yule (1911, p. 260), which requires the harmonic mean of the sample sizes.

TABLE 40
*The probability of certain differences of percentages of mutant types considered
 as deviations of simple sampling.*

Problem	Probability			
	Assuming one population (zero as most probable true difference)		Assuming two populations (sample difference most probable true difference)	
	Chance of deviation	Odds against deviation	Chance of deviation	Odds against deviation
Good vs. poor germination:				
With smooth type	.2646	2.8 : 1	.1083	8.2 : 1
With large type	.5466	.8 : 1	.2724	2.7 : 1
Same, $f_o = \sqrt{f_1 f_2}$.4910	1.0 : 1	.2445	3.1 : 1
With erenate type	.0320	30.25 : 1	.0066	150.5 : 1
Same, $f_o = \sqrt{f_1 f_2}$.0064	155.25 : 1	.0008	1249.0 : 1
With slender type	.7682	.3 : 1	.3835	1.6 : 1
With all types000074670	13,391 : 1
Same, $f_o = \sqrt{f_1 f_2}$ ^a0000081239	123,093 : 1
Small vs. large seeds:				
With erenate type	.0046	216 : 1	.0020 ^b	499 : 1 ^b
"Extreme" vs. "ordinary" parents:				
With slender type	.1114	8.0 : 1	.0557	17.0 : 1
Same, $f_o = \sqrt{f_1 f_2}$.0180	54.6 : 1	.0090	110.1 : 1

^a That is, using for large and erenate the values given by this formula (see text).

^b If the actual standard deviation of the five differences is used, $P = .0006$ and the odds are 1666:1 (see text).

In the calculation the deviations are taken from zero, and with these small numbers of samples the percentages are not thrown into classes; it suffices, then, to square each number of "successes," divide by the corresponding total of individuals, add the quotients, and divide by the grand total of individuals, correcting this weighted mean squared deviation by subtracting the square of the weighted mean percentage (percentage of grand total). If s is the number of successes and n is the total number of individuals in the subsample, and M is the weighted mean percentage, then $M = \frac{\sum s}{\sum n}$, and

$$\sigma_{\text{per cent}} = \sqrt{\frac{\sum \frac{s^2}{n}}{\sum n} - M^2}.$$

Table 39 gives, for the most important comparisons of heredity percentages, the total number of progeny (N), the number of cultural groups or (with the first line for each type) the number of parents (f), the average size of the groups of progeny (\bar{n}), and the mean percentage of the mutant type (p). This serves as a summary of some of the most important statistical data already presented relating to the inheritance of these types, and also shows the basis of the remaining part of this table and of table 40. For comparison of actual and theoretical standard deviations the theoretical value has been calculated from the actual percentage as given in this table. For comparison of means (table 40) the percentage of the corresponding total (p_0) has also been used, this theoretical standard deviation being the second in the table in the cases where the two values are not identical.

Since small changes in a percentage have little effect on its theoretical standard deviation, we are fairly well justified in taking the latter, as calculated from the actual percentage in each case, to be the "population" value. Consequently, the difference between the theoretical and actual standard deviations has been expressed in each case as a multiple of the probable error of the theoretical value.

Aside from the last line for crenate-leaved, where there is an obvious artificial reason for high variability, there is no very significant difference except with slender. In this case, the deviation of 5.7 times the probable error (line 1) is probably largely due to the genetic differentiation of "extreme" and "ordinary" parents suggested by their appearance and by the wide difference in the heredity percentages; the differences become moderate when the progeny of the two classes of parents are separated.

In the two cases (smooth-leaved and crenate-leaved types) where the percentages of mutant types differ greatly with good and poor germination, separation according to germination gives a mean value of the standard deviation decidedly lower than the value for all lots taken together. In the case of the large-leaved type there is little change, while the considerable reduction with the slender type is probably due to unequal separation of lots from parents genetically different.

Table 40 shows the simple-sampling probability of the most striking differences of heredity percentages, aside from the characteristic differences between different types. "Student's" (1917) table of probabilities of mean deviations with small samples is used, with interpolation by second differences. Where the standard deviation of the difference is required it is found from the theoretical values given in table 39 by the formula (Yule, 1911, pp. 264-265)

$$\sigma_{\text{difference}} = \sqrt{\sigma_1 + \sigma_2} = \sqrt{\frac{p_0 q_0}{n_1 - 3} + \frac{p_0 q_0}{n_2 - 3}},$$

when one statistical population is assumed (table 40, columns 2 and 3). When two populations are assumed (table 40, columns 4 and 5) the corresponding formula using $p_1 q_1$ and $p_2 q_2$ is employed. In the one case where this is possible (the seed-size test), it is also calculated from the actual differences of the pairs of percentages in the separate tests, each difference being weighted with the total number of progeny from the parent concerned. Where two values of f (the n of "Student's" table) are involved, the smaller is taken, giving understatements of the probabilities involved; in the two cases where the difference is more than 2, the values are recalculated, with f as the nearest smaller integer to the geometric mean of the two actual numbers (that is with $f_0 = \sqrt{f_1 f_2}$). In the case where the probabilities of four deviations all in the same direction are combined, the four chances of occurrence are multiplied together; that is, if the $\frac{1}{2}(1 + a)$ of "Student's" table is P , and $1 - P$ is F , then $F_{1,2,3,4} = F_1 \cdot F_2 \cdot F_3 \cdot F_4$.

"Student" (1908, p. 1) says, "The usual method of determining the probability that the mean of the population lies within a given distance of the mean of the sample, is to assume a normal distribution about the mean of the sample . . ." When this is done with a difference of means, it is at once evident that only half of the chances of deviations as great as the distance of the given difference from zero difference lie below zero difference; the other half of the chances of

such deviations lie in the opposite direction and represent positive differences still greater than the sample difference. In other words, if the implications of a sample difference are to be given full weight, this difference must be considered the *most probable value* of the theoretical "true" difference between *two assumed distinct statistical populations*. In the present case we wish to know the probability that the "true" or theoretical-population means differ in the same sense as the observed sample means. This involves calculation of the probability of deviations in one direction (beyond zero difference) from the sample difference. If the sample difference of means is considered as positive, then the negative "tail" of the theoretical frequency curve of sample differences (this curve being centered at the observed sample difference) must be compared with the rest of the curve. The positive portion of the curve the $\frac{1}{2}(1+a)^{24}$ of the tables, then gives the chances favoring the hypothesis that the sample means truly represent the population means. The odds in favor of the hypothesis are therefore given by the formula

$$O_1 = \frac{\frac{1}{2}(1+a)}{\frac{1}{2}(1-a)} \text{ or } \frac{\frac{1}{2} + \frac{1}{2}a}{\frac{1}{2} - \frac{1}{2}a}.$$

Values calculated from this formula are given in columns 4 and 5 of table 40.

When other considerations than the sample evidence are to be taken as determining the most probable value of the "true" mean, the case is different. For example, if the probability that our sample percentages are mere sampling deviations from some theoretical Mendelian value were in question, that theoretical value must be taken as the population mean and only the magnitude of the deviations must be considered.

When a difference of means is considered from this latter standpoint, it is assumed that the two samples come from *one statistical population*, and hence that zero is the most probable value of the population difference. If we choose to assume that the most probable value of the population difference in our cases is zero, we must calculate the odds against a deviation of the observed amount in either direction from zero difference. The formula for these odds is

$$O_2 = \frac{\frac{1}{2}(1+a) - \frac{1}{2}(1-a)}{2 \times \frac{1}{2}(1-a)} \text{ or } \frac{a}{1-a}.$$

²⁴ The whole area of the frequency curve is taken as unity, and a is the area enclosed by any given deviation in both directions from the mean.

Values from this formula are given in columns 2 and 3 of table 40; their magnitude in three cases, however, and the uniform agreement of the direction of difference with the expectation from biological evidence which has been discussed, weigh heavily in each test against the assumption of random sampling from a single statistical population.

It does not appear necessary, however, thus to weigh the evidence in detail before deciding which formula is suited to the case. There is no evident theoretical value from which these percentages are reasonably likely to be *sampling* deviations. This being the case, and granting such general possibilities as that of differential viability, it seems most reasonable to use the former (0_1) formula. That is, we should give full weight to the implications of a sample deviation unless there is some definite reason for assuming that some other value better represents the mean of the theoretical statistical population.

It must be remembered that the actual probabilities of sampling deviations do not necessarily correspond closely with the probabilities of *random* sampling. With the material in table 40, however, aside from the germination comparison in the case of the slender type, table 39 suggests a fair agreement with the conditions of random sampling. The actual standard deviations of the subsamples do not in general differ widely from the corresponding theoretical values, and the differences are negative about as often as positive.

The hypothesis of selective elimination with poor germination is strongly sustained (table 40), although only one difference (with the crenate type) has much statistical significance when considered alone. If we may multiply together the members of the four ratios in column 3 of the table, the combined odds (using the f_0 values) are 130:1 against occurrence of these four deviations as accidents of simple sampling, when magnitude of deviation alone is considered. If direction of deviation alone is considered the random chance of these four deviations all in the same direction is obviously $(\frac{1}{2})^4$, or the odds favoring the elimination hypothesis are 15:1. Combination of these two chances indicates a high probability for the hypothesis. When the two-population formula is used in calculating the standard deviation of the difference (columns 4 and 5) the value of P is considerably reduced in some cases, and the combined odds obtained from $F_1 \cdot F_2 \cdot F_3 \cdot F_4$ are very high. Evidently the best single expression of the simple-sampling odds, though possibly somewhat too high, is the value given last in column 5, or 123,093:1.

With the seed-size test of crenate the odds are 499:1 with the theoretical standard deviation of the difference, or 1666:1 with the

actual standard deviation. When the relatively small size and weak growth of crenate seedlings are also taken into account, the relatively small average size of crenate embryos may be considered to be demonstrated beyond reasonable doubt.

With "extreme" and "ordinary" slender parents the odds decidedly favor the hypothesis of genetic differentiation of parents, in spite of the small numbers involved. We must remember that definite statistical differentiation of lots of progeny grown under uniform conditions does not necessarily demonstrate *genetic* differences (differences in output of gametes) between the parents; in this case, however, the difference in the appearance of the parents and in the single-double ratio among the progeny also suggest genetic differentiation.

GENERAL DISCUSSION²⁵

It might be argued with some plausibility that the available evidence hardly justifies conventional factorial analysis, or at least that the data indicate strongly the presence of marked factorial inconstancy. The aberrant types occur in very small proportions among the progeny of selfed Snowflake parents, in much larger proportions from "mutant-type" parents, and in intermediate proportions from crosses with Snowflake. It might be supposed that the Snowflake type has a slight tendency to mutate to the other types, and that these have a much more marked tendency to mutate back to Snowflake. Various considerations, however, especially the occurrence of apparently regular linkage phenomena, seem to favor the general form of hypothesis which has been presented.

As we have seen, it is well known from the behavior of various factors that the typical Mendelian mechanism is present in *Matthiola*. It cannot be argued here, as sometimes with *Oenothera*, that the genetic behavior of the genus or species is fundamentally non-Mendelian. Since the Mendelian mechanism is demonstrably present, and Muller's (1918) work on beaded wings in *Drosophila* seems to establish the adequacy of this mechanism in a closely parallel case, surely conventional factorial analysis should be carried as far as possible; in fact (Muller, 1918, p. 423), a Mendelian explanation should not be abandoned for anything short of positively contradictory evidence.

²⁵ Muller's (1918) complete report on the beaded-wing case in *Drosophila* appeared several months after the present paper had gone to the publisher. Certain conclusions given below, very similar to Muller's but not credited to him, were therefore reached independently.

In the *Drosophila* case just mentioned, the "principal" factor for the character in question is "dominant for its visible effect and recessive for a lethal effect," so that no pure beaded individuals appear among the progeny of beaded. The original race regularly gave progeny partly heterozygous beaded and partly homozygous normal, while after a long period of selection a true-breeding beaded race appeared. This latter form, it proved, fails to give normals not because of being duplex for beaded—it is still simplex—but because of its possession of another factor, known only by its lethal effect when homozygous, which is carried by the chromosome bearing the normal allelomorph of the factor for beaded. The locus of this recessive lethal factor gives in general about 10 per cent of crossovers with the locus of beaded, but in this case, because of the presence of a factor "which almost entirely prevents crossing over" between the loci of the two lethal factors, viable non-beaded zygotes are very rarely produced. Thus every zygote receiving either two beaded-carrying chromosomes or two non-beaded-carrying chromosomes of the pair concerned fails to develop, and all the insects produced are necessarily heterozygous for both lethal factors.

A point of special interest in this case is the fact that by certain crosses individuals can be produced which give certain types among their progeny in very small percentages. Muller suggests that part at least of the supposed mutants of *Oenothera* may be due to crossing over between chromosomes carrying lethal factors, by which certain recessive factors are permitted to come to expression in viable zygotes.

For the inheritance of doubleness of flowers in *Matthiola* he gives a "balanced-factor" explanation essentially identical with mine (Frost, 1915).

There seems to be little reason to doubt that the differential factors for these aberrant *Matthiola* types have originated by mutation. On the analogy of *Drosophila* we might expect that the true mutations would be relatively rare, and that most of the apparent mutants, in cases where they appear frequently, would be due to segregation, appearing as the result of crossing over in chromosomes carrying balanced lethal factors. The evidence seems to indicate, however, that the differential factors for the mutant types at all extensively studied are dominant for their visible effects and usually (probably imperfectly) recessive for a lethal effect, the mutant factors thus being genetically similar to the factor for beaded wings in *Drosophila*. This would seem to imply the occurrence of certain mutations in pro-

portions as high as about 1 per cent, and a general mutation coefficient of perhaps 4.5 per cent, while the only Mendelian alternative would seem to be some more complex scheme whose satisfactory formulation might require much more extensive hybridization data.

To be more specific: (1) these types are not single recessives, since they are not homozygous but split into the mutant and "normal" types; (2) they are not simple cases of multiple recessives, as has been proposed by Heribert-Nilsson (1915) for *Oenothera* mutations, since what is on that hypothesis the full dominant type reappears with selfing; (3) if these types are single dominants, as they appear to be, they cannot (barring the action of inhibiting factors) arise from the pure recessive "normal" or Snowflake type by segregation, but only by immediate mutation; (4) they are not simple cases of complementary dominant factors, since they occur among the progeny of selfed parents.

We might assume that a "mutant" type depends on two pairs of factors, one homozygous and the other heterozygous, while both pairs are heterozygous in the "mutating" Snowflake parent. Thus the crenate type might have the zygotic formula $\frac{D}{d} \frac{Ci}{ci}$, where d is the factor for double flowers, C a dominant factor for crenate, and I a dominant inhibitor of C , all three loci being situated in the same chromosome, at distances of, say, 16 and 4 units apart, in the order indicated. A Snowflake parent producing crenate progeny would then be $\frac{DCI}{dci}$ or $\frac{DCi}{dci}$, and crossover combinations would produce the apparently mutant crenate progeny. The crenate progeny would behave as heterozygous dominants when selfed, and if CC zygotes were non-viable would yield constant Snowflake and inconstant crenate; the extracted Snowflake singles, having the composition $\frac{Dci}{dci}$, could not throw crenate individuals except by true mutation of c to C . With selfed Snowflake, if we assume 16 per cent and 4 per cent of crossing over in the two positions, and a 60-per-cent selective elimination of crenate zygotes, all CC zygotes being non-viable, substantially the observed percentages of crenate singles and doubles result.²⁶

²⁶ See page 125, footnote. This scheme agrees fairly well with the results from crossing, and gives almost exactly the observed proportion of total doubles (a little over 53 per cent) for selfed Snowflake. Its adequate presentation must be reserved for a later paper.

Formerly (Frost, 1916) the hypothesis of frequent dominant mutations seemed the more probable, but there is apparently non-conformable evidence. It is true that the peculiar behavior of the slender type might conceivably depend on an occasional mutation in another locus, or an exchange (Shull, 1914) or duplication of loci, giving two similar or identical factors for slender. An apparently fatal objection, however, is the fact that the supposed mutants seem to show linkage with singleness or doubleness at their origin from Snowflake as well as in subsequent generations—a fact which strongly suggests segregation in the former case.

If the apparent mutants are really due to segregation complicated by lethal action, the origin of the complex heterozygosis indicated for Snowflake is doubtful; it may be due to hybridization, but more probably to a gradual accumulation of mutant factors in balanced-lethal combinations. On the analogy of Muller's *Drosophila* case, especially, it might be expected that the latter would be the true explanation, particularly since self fertilization seems to be the rule in *Matthiola*. On this basis the term *mutant type* is used with some confidence in this paper, while the aberrant individuals have been called *apparent mutants*.

We must not forget that some of the mutant types may arise, as with *Oenothera gigas* and *O. lata*, by non-disjunction, or reduplication of chromosomes, and that this fact may determine their heredity. This is not to be expected with the types whose factors show apparent coupling with singleness or doubleness, but it might be true of the apparently unlinked smooth-leaved type. A preliminary study of several types shows that the usual somatic number of chromosomes is probably fourteen, but that positive counts are difficult. While it might be very hard to demonstrate the regular presence of one extra chromosome in an individual or a type, it should be easy to decide between the diploid and triploid numbers. The large-leaved type is so strongly suggestive of *O. gigas* that it would not be surprising to find the triploid number in the material now on hand for examination.

In a preliminary paper on these types the writer (Frost, 1916) discussed some possible relations of mutation, heterozygosis, and partial sterility, with special reference to *Oenothera*, mentioning the possibility that special prevalence of heterozygosis in the genus may be, "in large part, a *result* rather than a *cause* of *mutation*." This suggestion is evidently justified even if much of the supposed mutation of *Oenothera* is really segregation, since it is highly probable that

the peculiar phenomena depend on lethal factors or combinations of factors originally due to mutation.

Another possibility there mentioned, advanced by Belling (1914) and since specially discussed by Goodspeed and Clausen (1917), is that of the occurrence of lethal *combinations* of certain factors which in other combinations may be in no way prejudicial to normal development. As the latter paper shows, it is probable that in certain crosses between "good species" most of the new combinations brought together in the formation of F_1 gametes are incompatible with the production of functional gametes. Perhaps in the case of *Oenothera* there may exist within a species factors lethal in any combination when homozygous, and other factors lethal only in certain combinations.

A balanced-factor explanation for the inheritance of doubleness²⁷ in *Matthiola*, a case which Muller (1918) discusses, seems to have been first definitely stated by Goldschmidt (1913), though he failed to provide for one feature of the evidence, the deviation of the heredity ratio from 50 per cent. As has been shown (Frost, 1915), this peculiarity may be due to greater viability of the homozygotes (sterile doubles) during embryonic development, since the doubles are more viable in the mature seeds and more vigorous in later development (Saunders, 1915). In this case the "normal" factor is completely eliminated in favor of the mutant (sterile-double) factor in the formation of the sperms, and probably is partially eliminated in the formation of either the eggs or the embryos or both.

Here the normal singleness (sporophyll) factor *D* may act as a lethal in the heterozygous parent, possibly from its general relations of growth vigor in the presence of the more vigorous *d*-carrying cells. If the lethal factor is situated in a distinct locus, evidently crossovers are at most extremely rare. It is true that Miss Saunders (1911) finds that F_1 hybrids with pure single forms produce functional single-carrying pollen; with the pure single forms from which the original "double-throwing" mutants arose, however, this might not be true, or a lethal change may have occurred in the singleness factor itself rather than in a factor coupled with it. The *Drosophila* case would suggest a lethal change in another locus of the single-carrying chromosome.

In my paper of 1915 this lethal change in one chromosome apparently accompanying the mutation of *D* to *d* in the homologous

²⁷ For a brief outline of the genetic behavior of doubleness see the discussion of the experimental data for the smooth-leaved type.

chromosome was considered puzzling. Evidently, however, it may have occurred in one chromosome before *D* mutated to *d* in the other, and even then may have produced its lethal effect. It is evident that if doubleness should arise in the absence of the lethal effect it would tend to be eliminated by the return of one-third of the singles to the homozygous condition in each generation. In fact, it is possible that the lethal change arose later than doubleness, as in the *Drosophila* case, or was brought in later by cross pollination, and happened to be preserved as a result of horticultural selection for a high proportion of doubles.

A parallel-column comparison between the double type and the types especially discussed above has already been given, in connection with the smooth-leaved type. It will now be seen that this comparison seems to apply to all mutant types, except early, that have been genetically tested, the principal differences between these types relating to the heredity percentage and the apparent presence or absence of linkage with the single-double factors.

From the standpoint of its relation to genetic analysis the doubleness factor is remarkably similar to the sex factor in animals. There are two types in each generation, one heterozygous and the other evidently homozygous, and these types are produced by the fertilization of two kinds of eggs, produced in equal or nearly equal numbers, by a single kind of sperm. Although one of the somatic types is sterile, and the uniformity of the sperms produced by the other is due (evidently) to lethal action, the opportunity for chromosome analysis is similar to that with sex chromosomes.

We may say that the doubleness factor and its normal allelomorph (*d* and *D*) are carried by chromosome pair I. Already we know several other pairs of factors evidently carried by this pair of chromosomes. These are, to name only the mutant or possibly mutant member of each pair of factors: *P* (pale sap color) and *W* (colorless plastids), both studied by Miss Saunders (1911, 1911*a*); *C* (crenate-leaved), *S* (slender; possibly two factors), and *N* (narrow-leaved). As we have seen, the last three of these are probably lethal when homozygous, and one or more unidentified lethal factors may be concerned in the breeding results, while the doubleness factor affects the race much like a recessive lethal, since all *dd* individuals are completely sterile.

SUMMARY

This paper describes the occurrence, characteristics, and heredity of certain aberrant plant types which decidedly resemble some of the "mutant" types produced by *Oenothera lamarckiana*. The parent form is *Matthiola annua* Sweet, of the horticultural variety "Snowflake."

These aberrant forms may be called mutant types, since it is highly probable that they are originally produced by mutation. The aberrant individuals may be termed apparent mutants, since it may be considered uncertain whether they usually arise by immediate mutation or by segregation. The case acquires special significance because individuals belonging to the mutant types, although the species is known to be typically Mendelian with respect to various characters, give erratic heredity ratios suggestive of *Oenothera*.

At least eight types have been somewhat carefully studied, and six of these have shown their heritability in progeny tests. Several other types have been named, but for various reasons their distinctness is more or less doubtful.

Some of the commoner types have each been produced by many parents, and in several pure lines isolated from the original commercial variety. The apparent mutants other than the early type compose about four or five per cent of the progeny of Snowflake and early parents, the separate types ranging down from about one per cent.

Most of the mutant types are in general inferior to Snowflake in vigor, and the difference in development is greatly increased by certain unfavorable environmental conditions. The proportion of apparent mutants in cultures from Snowflake parents appears to be definitely lower where germination is comparatively poor.

The mutant types differ from Snowflake and from each other in various respects. The early type is practically a smaller and earlier Snowflake. The other mutant types, on the other hand, differ markedly from Snowflake in vigor, fertility, and various form and size characters. Each type is named from some conspicuous characteristic difference from Snowflake, but usually various other differences can readily be found.

Somewhat extensive progeny tests have been made for five of the mutant types, and a little evidence secured for two or three other types.

The early type is probably due to a single dominant mutant factor segregating normally from the corresponding Snowflake factor; the quantitative nature of its differences from Snowflake, however, makes positive determination of this point a matter of great difficulty.

At least five other types plainly reproduce themselves, but about 50 to 70 per cent of the progeny are usually Snowflake; no true-breeding individual of any generation of any of these types has yet been tested. Genetic work with most of these types has been much hampered or even prevented by low vigor and fecundity, and the aggregate data from progeny of parents of four types strongly indicate selective viability at germination. It has been determined by crossing that in three of the types the mutant factor (or factors) is carried both by eggs and by sperms. From these facts it seems probable that homozygotes of the mutant types are non-viable, and that severe selective elimination occurs during embryonic development; or, in other words, that the mutant factor is imperfectly recessive for a lethal effect.

In three types there appears to be linkage with the factor pair for singleness and doubleness of flowers, the mutant factor being coupled with singleness in the tested apparent mutants of two types, and with doubleness in the third type. With two other types these factors seem to be independent. No reversal of coupling has been found in later generations of the former two types, but on the scheme presented crossover singles should be scarce.

For one type (crenate-leaved) a hypothesis based on the facts stated gives very closely the ratio obtained from selfed parents. Reciprocal crosses with Snowflake conform less closely to the requirements of the hypothesis, but do not definitely contradict it. The slender type, which shows similar apparent linkage, seems to disagree definitely with the hypothesis; there is strong evidence, however, that slender individuals may differ genetically among themselves.

A more complex scheme providing also for the usual origin of these types from Snowflake by segregation is briefly outlined.

The selfing ratios are very suggestive of duplication of a chromosome (non-disjunction), as in *Oenothera lata*, but it is hard to reconcile the cases of apparent linkage with this hypothesis. It seems probable that these three linked types have originated and are transmitted in the same general way as the double-flowered type, and that all of these four mutant factors (including double) represent changes of some sort within a chromosome of the same pair, which may be

numbered I. Miss Saunder's work shows that two flower-color factors also belong to this linked group.

The large-leaved type strikingly resembles *Oenothera gigas*, and it may prove to be triploid in nuclear constitution. In that case segregation may be irregular and genotypically intermediate individuals may be more or less frequently produced.

It is probable that further study of these types will help to explain the remarkable genetic behavior of *Oenothera* and of *Citrus*.

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PLATE 22

THE EARLY TYPE

Fig. 1. March 20, 1908. The single progeny of WG9. Plants from house M to the reader's left from stake, from house W to right of stake, from house C below. WG9-C10, the early apparent mutant, is the middle plant in the lower row. The stake indicates inches.

Fig. 2. About May 1, 1908. WG9-C10 at the left, WG9-C9 (Snowflake) at the right.



Fig. 1



Fig. 2

PLATE 23

THE EARLY TYPE

Fig. 3. April 8, 1909. The single progeny of WG9-C9 (Snowflake); arrangement as in figure 1.

Fig. 4. April 9, 1909. The single progeny of WG9-C10 (heterozygous early). Warm-house plants partly at right of stake in lower row; arrangement otherwise as in figure 3. Compare with figure 3, house by house.

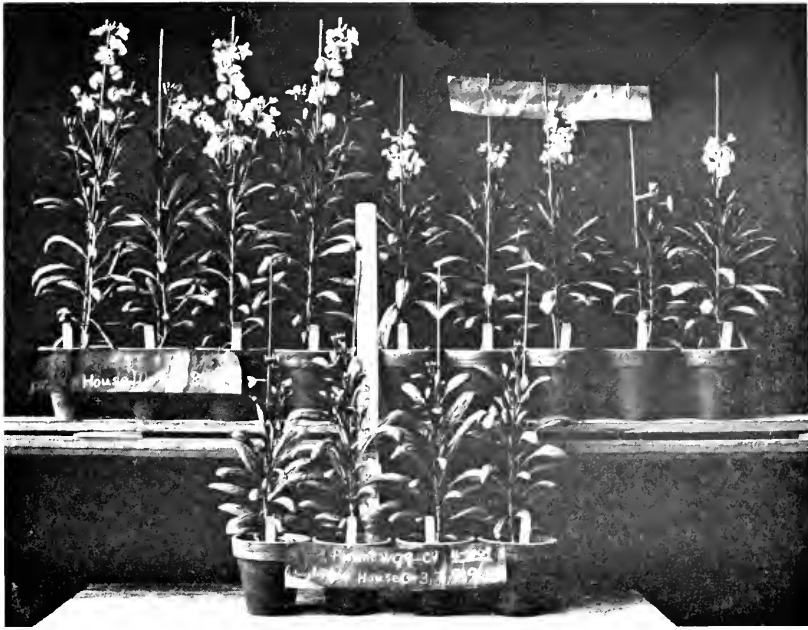


Fig. 3



Fig. 4

PLATE 24

THE EARLY TYPE

Fig. 5. July 19, 1911. Lots 1 to 10, with lots 11 to 14 mostly in sight at the right. Odd-numbered lot in nearer (west) half of each row.

Fig. 6. July 19, 1911. Lots 19 to 28, with lots 15 to 18 mostly in sight at the left.



Fig. 5



Fig. 6

PLATE 25

THE SMOOTH-LEAVED TYPE

Fig. 7. April 27, 1909. Smooth-leaved apparent mutants. Compare with figures 3 and 4 as to earliness, noting the difference in date.

Fig. 8. May 29, 1914. Progeny of a smooth-leaved parent. Plant at right Snowflake single, the others smooth.

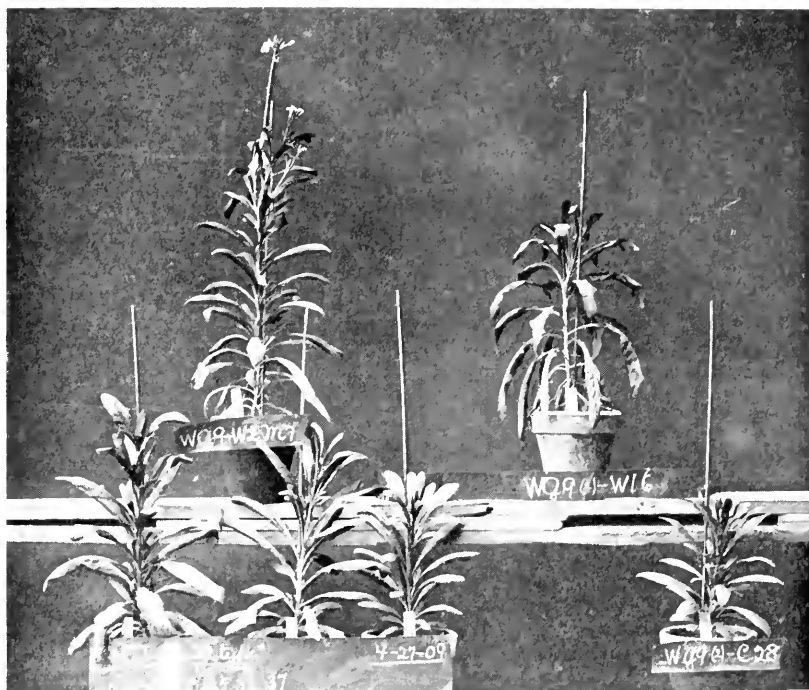


Fig. 7



Fig. 8

PLATE 26

THE SMOOTH-LEAVED TYPE

Fig. 9. June 28, 1915. Progeny of a smooth-leaved parent. Smooth single at left, Snowflake double at right.

Fig. 10. Same date and parent as with figure 9. From left to right: Snowflake double (also shown in figure 9), Snowflake single, smooth double.



Fig. 9



Fig. 10

PLATE 27

THE LARGE-LEAVED TYPE

Fig. 11. August 29, 1914. Progeny of a large-leaved parent (28a), near the close of the hot Riverside summer. From left to right: large single, large double, Snowflake single (two, the first injured by aphids).

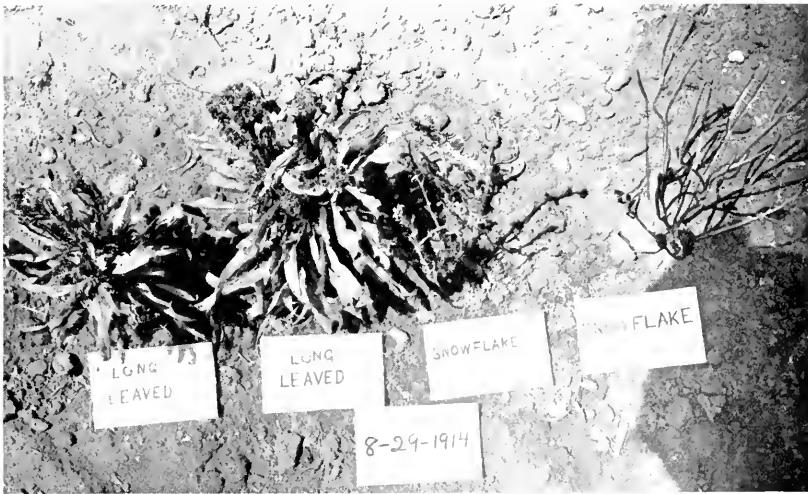


Fig. 11

PLATE 28

THE LARGE-LEAVED TYPE

Fig. 12. July 8, 1916. Progeny of a large-leaved parent. Middle plant Snowflake; the rest large; all single.

Fig. 13. July 8, 1916. Progeny of a large-leaved parent, more than a month older than those shown in figure 12. From left to right: large double, Snowflake double, large single.



Fig. 12



Fig. 13

PLATE 29

THE CRENATE-LEAVED TYPE

Fig. 14. April 6, 1909. Crenate-leaved apparent mutants. Note the variation in leaf serration, and especially the slightness of the serration (or crenation) with the one cool-house plant (below).

Fig. 15. April 14, 1911. Progeny of a crenate-leaved parent, grown in a cool greenhouse. The first two plants at the right are Snowflake, the rest crenate.



Fig. 14



Fig. 15

PLATE 30

THE CRENATE-LEAVED TYPE

Fig. 16. July 8, 1916. Progeny of a crenate-leaved parent. From left to right: crenate single (two), crenate double, Snowflake double.

Fig. 17. July 8, 1916. Snowflake \times crenate-leaved, F_1 . From left to right: smooth, Snowflake single, crenate double (two).



Fig. 16



Fig. 17

PLATE 31

THE SLENDER TYPE

Fig. 18. April 27, 1909. Miscellaneous aberrant individuals, with two typical Snowflake plants (third from the left above, second from the left below). In upper row: second from left, narrow double; second from right, slender double. In lower row at left, slender single (25b).

Fig. 19. April 14, 1911. Progeny of a slender parent (25b). Two at the right Snowflake, the rest slender.

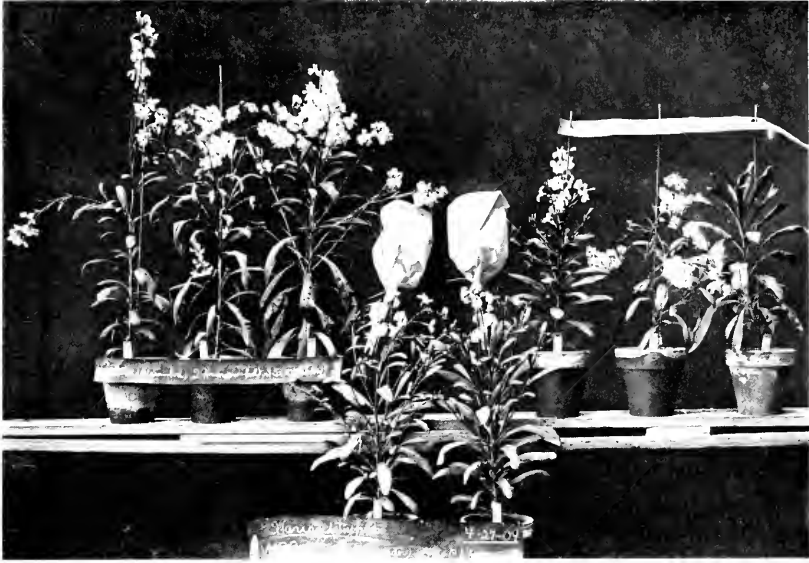


Fig. 18



Fig. 19

PLATE 32

THE SLENDER TYPE

Fig. 20. June 3, 1914. Progeny of slender parents. From left to right: slender single, slender double, Snowflake double.

Fig. 21. July 7, 1916. Snowflake \times slender, F₁. Middle plant Snowflake; the others slender; all single.



Fig. 20



Fig. 21

PLATE 33

THE NARROW-LEAVED TYPE

Fig. 22. April 13, 1911. Narrow-leaved apparent mutants.

Fig. 23. June 3, 1914. A narrow-leaved apparent mutant among progeny of a crenate-leaved parent. From left to right: narrow double, crenate single (two).

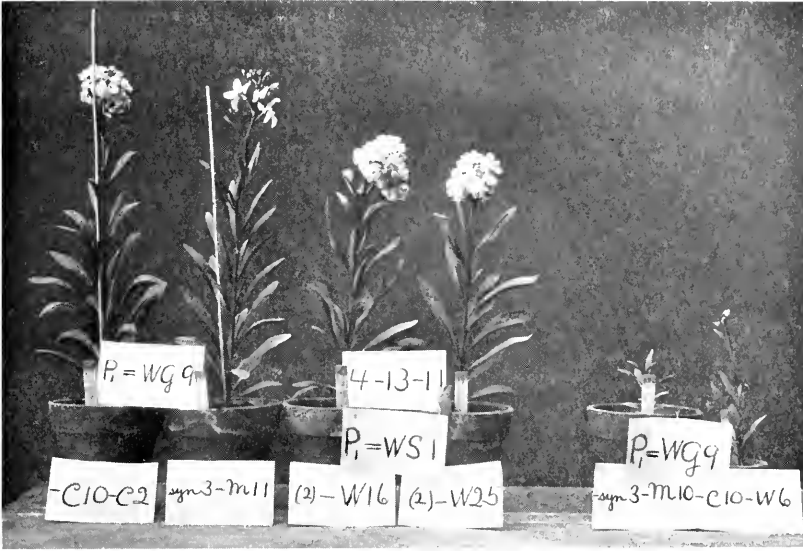


Fig. 22



Fig. 23

PLATE 34

THE NARROW-LEAVED AND SMALL-SMOOTH-LEAVED TYPES

Fig. 24. June 28, 1915. A narrow-leaved apparent mutant among F_1 progeny from Snowflake \times slender. Narrow double at left; the rest Snowflake single.

Fig. 25. April 14, 1911. Miscellaneous aberrant plants, some being apparent mutants. From the left: first and fifth small-smooth, third stout dwarf, seventh slender. See text.



Fig. 24



Fig. 25

PLATE 35

THE NARROW-DARK-LEAVED TYPE

Fig. 26. June 3, 1914. A narrow-dark-leaved apparent mutant among progeny of a narrow-leaved parent. Third plant from left narrow-dark single; the other three Snowflake double.

Fig. 27. June 28, 1915. Progeny of a "small-convex-leaved(?)" parent (27a). From left to right: narrow-dark single, Snowflake double, smooth single.



Fig. 26



Fig. 27

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Vol. 2, No. 5, pp. 191-204, plates 36-38

October 19, 1920

INTERSPECIFIC HYBRIDS IN CREPIS

I. CREPIS CAPILLARIS (L.) WALLR.
× C. TECTORUM L.

BY

ERNEST B. BABCOCK AND JULIUS L. COLLINS

UNIVERSITY OF CALIFORNIA PRESS
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ERNEST B. BABCOCK AND JULIUS L. COLLINS

Although the problem of the mechanism of heredity may be said to have been solved by Morgan and others¹ by means of the genetic analysis of a species of flies, *Drosophila melanogaster*, there yet remains the highly important question regarding the generality of the conclusions based on the *Drosophila* data. As has been pointed out by Morgan,² no method of determining the specific relation of individual chromosomes to particular somatic characters appears more promising than the study of hybrids between species. Especially is this the case when the species possess low chromosome numbers. If such species can be subjected to extensive genetic analysis so that the factorial composition of each pair of chromosomes may be described with some degree of exactness, and if fertile hybrids between such species can be obtained so as to permit of breeding as well as of cytological investigations of the hybrid progeny, we should find here the most promising material with which to test the generality of the chromosome theory of heredity.

Inasmuch as several species of *Crepis* were known to possess low chromosome numbers, considerable attention has been given by the writers³ to the genetic investigation of two of these species, viz., *Crepis capillaris* (*virens*)⁴ and *C. tectorum* L., the results of which

¹ Morgan, T. H., Sturtevant, A. H., Muller, H. J., and Bridges, C. B., *The Mechanism of Mendelian Heredity*. New York, Holt, xiii + 262 pp., frontispiece, and unnumbered diagrams. 1915.

² Morgan, T. H., *The Physical Basis of Heredity*. Philadelphia, Lippincott, pp. 1-305, 117 illustrations. 1919.

³ Babcock, E. B., "Crepis—a promising genus for genetic investigations." *Amer. Nat.*, vol. 54 (1920), pp. 270-276.

⁴ Britten, James, and Rendle, A. B., "Notes on the 'List of British Seed Plants.'" *Jour. Bot.*, vol. 45 (1907), p. 102.

will be reported later. But before confining our attention too largely to one or two species only, it was deemed advisable to look into the possibility of obtaining fertile hybrids between these species.

Although the species in question show differences in many morphological characters and in at least one physiological character, only those having to do with the seedling stage will be considered here, due to the fact that the hybrids in all cases died during this stage.

The achenes of *Crepis capillaris* range in length between 2.0 and 2.5 mm.; they have no beak, and the pappus sheds rather easily. The cotyledons vary from broadly ovate to the condition where the breadth of the widest part is greater than the length, in which case the tip of the cotyledon is distinctly dentate. The first plumule leaf makes its appearance very quickly after the cotyledons have expanded to their normal size. The cotyledons are approximately 5 mm. wide and 4 to 6 mm. long (pl. 36, fig. 2).

The achenes of *Crepis tectorum* range from 3.5 to 4 mm. in length and are correspondingly thicker than the *capillaris* achenes. The *tectorum* achenes are also beakless but they retain their pappus more persistently than do those of the other species. The cotyledons are distinctly different in both shape and size. The general shape is narrowly linear with bluntly pointed ends. Length of cotyledons varies around 6 mm., the width around 3 mm. As in *capillaris*, the plumule leaves appear very promptly, usually one at a time, but occasionally in both species two plumule leaves appear simultaneously. Thus there is evident a distinct difference in size and shape of the first or cotyledon leaves of the two species corresponding with the difference in size of achenes, and a resemblance in the prompt appearance of the plumule leaves (pl. 36, fig. 1).

Crepis tectorum possesses one more pair of chromosomes than *C. capillaris*, the former having four pairs, the latter, three pairs (pl. 38, fig. 2).

Two methods of pollination were employed, which will be described in detail in another paper: (1) Emasculation of female parent flowers; (2) female parent not emasculated but washed free of its own pollen by use of a fine jet of water. The second method was used when the *capillaris* plant had given indications from selfing tests of being self-sterile. The results of cultures thus secured are indicated below. The female parent is mentioned first in each cross.

Culture Number	Parents	Cotyledon Characters of Seedlings	Method of Pollination	Behavior of Hybrids	Number of Seedlings
Z 3	<i>capillaris</i> × <i>tectorum</i>	6 <i>tectorum</i> 5 <i>capillaris</i> 1 intermediate	2	All failed to pass cotyledon stage	12
Z 5	<i>capillaris</i> × <i>tectorum</i>	All <i>tectorum</i> , showed hybrid vigor	2	All failed to pass cotyledon stage	6
Z 7	<i>capillaris</i> × <i>tectorum</i>	All <i>tectorum</i> , showed hybrid vigor	2	All failed to pass cotyledon stage	3
Z 8	<i>capillaris</i> × <i>tectorum</i>	Intermediate	2	All failed to pass cotyledon stage	1
Z 10	<i>tectorum</i> × <i>capillaris</i>	All <i>tectorum</i> , showed hybrid vigor	1	All failed to pass cotyledon stage	12
Z 12	<i>capillaris</i> × <i>tectorum</i>	Small and distorted, abnormal	1	Failed to pass cotyledon stage	1
Z 13	<i>capillaris</i> × <i>tectorum</i>	Small and distorted, abnormal	2	All failed to pass cotyledon stage	5

Z 3 F₁ (18.42 P₂₁ × p2 P₅₁) CAPILLARIS × TECTORUM

Three heads from which pollen had been washed with a jet of water were then pollinated with *tectorum* pollen and covered with a bag. These heads produced forty-two achenes which were smaller even than the average *capillaris* achenes. Of twenty-four placed in the germinator, thirteen sprouted and the twelve surviving divided themselves in the cotyledon stage into three groups. Six appeared like *tectorum* seedlings, five like *capillaris*, and one was intermediate. These all died at the end of the cotyledon stage.

Z 5 F₁ (18.58 P₄ × p2 P₁₀) CAPILLARIS × TECTORUM

Achenes from this cross were planted at two different times. Of the four placed first in the germinator, only two sprouted, both having large *tectorum*-like cotyledons. The plumule leaves started on one

plant but failed to appear on the other. After remaining alive, but not growing, for eighty-one days the plant with the rudimentary plumule died. The one failing to produce even the rudimentary plumule leaves lived for a shorter period.

After this unsuccessful attempt, twelve remaining achenes were placed in the germinator. At the end of five days five had sprouted, four of which showed robust, healthy cotyledons which resembled those of *tectorum* seedlings but on an enlarged scale. They were essentially as the first two plants secured from the same lot of seed. After having produced abnormally large cotyledon leaves, and in some cases rudiments of plumule leaves, all the seedlings began to turn yellowish, and, despite efforts to revive or stimulate them, continued to decline until finally they died.

One of the plants which was beginning to show signs of distress was carefully removed from the soil by washing in a pan of water. The root was one inch long and had a blunt rounded tip covered by the rootcap. All along the root from the tip to the ground surface line were small knots or wartlike protuberances as if lateral roots might have been attempting to push out. Later cytological examination showed this to be the case. The root and the cap were turning brownish as if growth had ceased and decomposition had begun, although the above-ground parts had only begun to show signs of unhealthiness. The fifth plant of this culture was smaller than the others but otherwise like them.

When the plants began to show symptoms of declining health some of them were treated with ether in an effort to stimulate them to new growth, but this appeared to have no effect and all perished.

Z 7 F₁ (18.204 P₆ × p2 P₅₁) CAPILLARIS × TECTORUM

Eight F₁ achenes were produced. Three out of five achenes produced seedlings which showed their hybrid nature by developing large *tectorum*-like cotyledons, by their failure to produce plumule leaves, and by their inability to pass beyond the cotyledon stage.

Z 8 F₁ (18.201 P₁ × p2 P₅₁) CAPILLARIS × TECTORUM

Only one achene was produced on one head washed free of its own pollen and pollinated with *tectorum* pollen. This achene produced a seedling which appeared to be intermediate between its parents, and like other hybrid seedlings died at the end of the cotyledon stage.

Z 10 F₁ (p2 P₅₁ × e4 P₂) TECTORUM × CAPILLARIS

This represents the reciprocal of the above mentioned crosses in which *capillaris* was used as the female parent. All *tectorum* flowers used in hybridization work were emasculated in the bud stage.

Six F₁ seedlings were secured, all exhibiting *tectorum* cotyledons on an enlarged scale. All died at the end of the cotyledon stage, some having started to produce plumule leaves which resulted only in rudimentary and abnormally shaped structures too small to be described in detail. Ether treatment of two slowly dying seedlings failed to stimulate them to renewed growth. These plants remained alive in the cotyledon stage thirty days.

Z 12 F₁ (18.42 P₁ × p2 P₅₁) CAPILLARIS × TECTORUM

In this culture *capillaris* flowers were emasculated in the bud stage before the stigma was receptive. Three heads produced four achenes, only one of which sprouted. It produced a small plant with undersized distorted cotyledons and no plumule. This weak seedling died in the cotyledon stage.

Z 13 F₁ (e14 P₇ × 212 P₂₁) CAPILLARIS × TECTORUM (KEW)

One washed head produced eight achenes. Three sprouted, and the plants had enlarged cotyledons which persisted for some time. One seedling produced several abortive plumule leaves but they all stopped growing when about 4 or 5 mm. long. It appeared unable to produce typical plumule leaves. Those formed were tiny threadlike structures and not at all like plumule leaves of normal seedlings. The diameter of this plant at sixty days was three fourths of an inch. (Some of the normal *tectorum* plants produce seed in ninety days.) Another seedling went through essentially the same process and died when four months old. The third plant had twisted, deformed cotyledons, and each appeared to have a separate root. They were separated, each cotyledon placed in a pot of soil, where one died after four days, the other continuing the struggle for thirty-six days before it too perished.

It will be noted that cultures Z 10 and Z 12 are reciprocal crosses in which each female parent was emasculated, thus insuring hybridity, and that the behavior of the resulting seedlings was similar. The plants of both cultures failed to develop past the stage in which the

young seedling is nourished from the food material stored in the seed. Apparently in the combination of *capillaris* and *tectorum* the germinal elements are incapable of interacting in such a way as to cause the seedling to develop normally (pl. 36, fig. 3).

In a number of cases (not listed) where the *capillaris* female parent was washed and pollinated with *tectorum* pollen, a number of achenes were secured which germinated well, producing seedlings which appeared and behaved in every way like typical *capillaris* plants. These did not stop growth at the end of the cotyledon stage but continued normal development. They were maternal in all respects. Thus we get two kinds of results when depollination by water is substituted for emasculation, and *tectorum* pollen applied: (1) plants which show the *tectorum* type of cotyledon on an enlarged scale, and which die at the end of the cotyledon stage of development; (2) plants which show maternal inheritance and are able to develop past the cotyledon stage, the limit of development in class one. Class two occurs only when *capillaris* is the female parent and the water method of depollination is used. Of eleven crosses where the female was depollinated by means of the water jet, six produced F_1 seedlings having *tectorum* cotyledons on an enlarged scale, and all six failed to develop beyond the cotyledon stage; five produced F_1 seedlings typically *capillaris* which developed normally into *capillaris* plants.

From the evidence furnished by the equivalent results of reciprocal crosses when the female plant was emasculated (Z 10 and Z 12), we are led to conclude that seedlings of the second class described above, exhibiting maternal inheritance, were the result of self-fertilization of the *capillaris* plant, which may have occurred before washing or because of incomplete removal of the pollen by the water method, and that those of the first class, showing dominance of *tectorum* in F_1 and the failure to continue development, were true hybrids. As a check a number of heads were depollinated with water and bagged without pollination. In one case selfed seeds were produced in a bag covering heads so treated. This indicates that the method is responsible for the appearance of the *capillaris* plants where crossing was attempted. In no case were achenes produced on heads which had been emasculated and bagged without pollination as checks.

The above conclusions were confirmed by cytological examination. Cells from the mature plants (Z 9 P_6) were found to contain six chromosomes, the typical number for *capillaris*. Cells of the root tips from young seedlings of the hybrid class (Z 5) were found to contain

seven chromosomes, the sum of the haploid numbers of *capillaris* and of *tectorum*. Nothing can be learned of the reduction division because the plants never reached maturity, but there seems to be no difficulty in somatic division, all seven of the chromosomes dividing in an apparently normal fashion.

Examination of a young F_1 seedling (Z 5) which had reached the limit of development, revealed a most unusual teratological cell condition (pls. 37 and 38). The tissue systems of the plant were in a chaotic condition. Patches of embryonic tissue were distributed here and there among the larger vegetative cells, patches or sections of tracheary cells were likewise distributed here and there throughout the mass. Groups of vegetative cells were separated by streaks of disorganized and disintegrated tissue. It appeared as if the force that directs the organization and coördination of cell systems, whatever it is, was lacking. This lack of order in the cell systems prevented the functioning of these systems and caused cessation of development.

The principal features of the interspecific hybrids here recorded are:

1. Reciprocal crosses are equivalent.
2. F_1 shows dominance of *tectorum* cotyledon characters and hybrid vigor, as expressed by the increased size of the seedling parts.
3. Absence of complete organization and coördination of the functioning systems, which absence causes the death of the plant at the end of the cotyledon stage.

The possible origin of species having a larger chromosome number from species having a smaller number by fragmentation or segmentation of some of the latter has been suggested a number of times. Metz⁵ shows a diagrammatic gradation of chromosome numbers for different species of *Drosophila*. Hance⁶ applies the same idea to the origin of chromosome number variations in *Oenothera* species. Rosenberg⁷ recently concluded that the origin of *Crepis* species with three, four, and five pairs of chromosomes could best be explained by non-disjunction occurring during the reduction division. Bridges⁸ actually

⁵ Metz, C. W., "Chromosome Studies in the Diptera, I. A preliminary study of five different types of chromosome groups in the genus *Drosophila*," *Jour. Exp. Zool.*, vol. 17 (1914), pp. 45-59.

⁶ Hance, R. T., "Variations in the number of somatic chromosomes in *Oenothera scintillans* de Vries," *Genetics*, vol. 3 (1918), pp. 225-261.

⁷ Rosenberg, O., "Chromosomenzahlen und Chromosomendimensionen in der Gattung, *Crepis*," *Arkiv för Botanik*, Bd. 15 (1918), p. 11.

⁸ Bridges, C. B., "Non-disjunction as proof of the chromosome theory of heredity," *Genetics*, vol. 1 (1916), pp. 1-52 and 107-163.

found a female *Drosophila melanogaster* with five pairs of chromosomes which originated after secondary non-disjunction in both parents.

Assuming that *Crepis tectorum*, a species with four pairs of chromosomes, originated by non-disjunction of one pair of the *capillaris* chromosomes, we would expect a cross between these two to be compatible inasmuch as the chromosome complex should be identical, *tectorum* merely having one of the *capillaris* chromosomes in duplicate.

The demonstrated inability of hybrids between the two species to function normally leads to the conclusion that *Crepis tectorum* is not related in such a direct way to *Crepis capillaris*.

The results reported here indicate the desirability of making preliminary experiments in hybridizing all the species of *Crepis* that give promise of being of value for genetic investigations. Experiments with other species are now under way.

Grateful acknowledgment is made to Dr. Ruth F. Allen for her assistance in preparing the cytological material.

EXPLANATION OF PLATES

PLATE 36

Crepis seedlings. $\times 2$.

Fig. 1. *Crepis tectorum*. Normal seedling showing elongated cotyledons extending horizontally from the center. Note the two plumule leaves at right angles to the cotyledons.

Fig. 2. *Crepis capillaris*. Normal seedling showing short rounded cotyledons extending horizontally, one plumule leaf showing at right angles to the cotyledons.

Fig. 3. F_1 hybrids, *C. tectorum* ♀ \times *C. capillaris* ♂ (above). F_1 hybrids, *C. capillaris* ♀ \times *C. tectorum* ♂ (below). Seedlings show stage at which development ceases.

Fig. 4. Selfed seedling resulting from crossing method No. 2. Note the roundish *C. capillaris* type of cotyledons (marked *c*) not at all like those of the F_1 hybrids. Five plumule leaves are shown, the plant being the same age as the hybrids.

Fig. 5. F_1 hybrid seedling produced by crossing method No. 2. Notice the two small abnormal plumule leaves between the cotyledons. This shows the highest stage to which any of the hybrids developed.



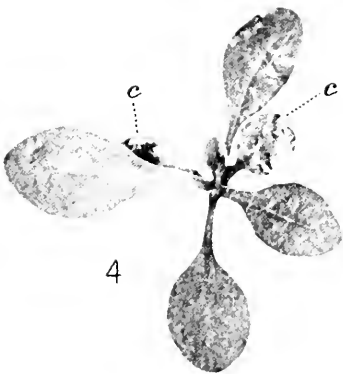
1



2



3



4



5

PLATE 37

Teratological tissue of F₁ hybrid *Crepis capillaris* × *C. tectorum*. Z 5.

Fig. 1. A vertical, not quite median, longitudinal section of the abortive plumule of a seedling. × 530.

a. An isolated patch of tissue surrounded by the slime (dark in reproduction) of disintegrating cells. Within the patch are the leaf tracheids and an irregular mass of meristematic dividing cells.

b. Above the main patch of meristem is a second smaller layer, also lying free in a layer of slime. It is about ten cells long and two or three cells thick. The cells nearest it on all sides are fully matured parenchyma.

c. Side section of apex of plumule showing only mature cells.

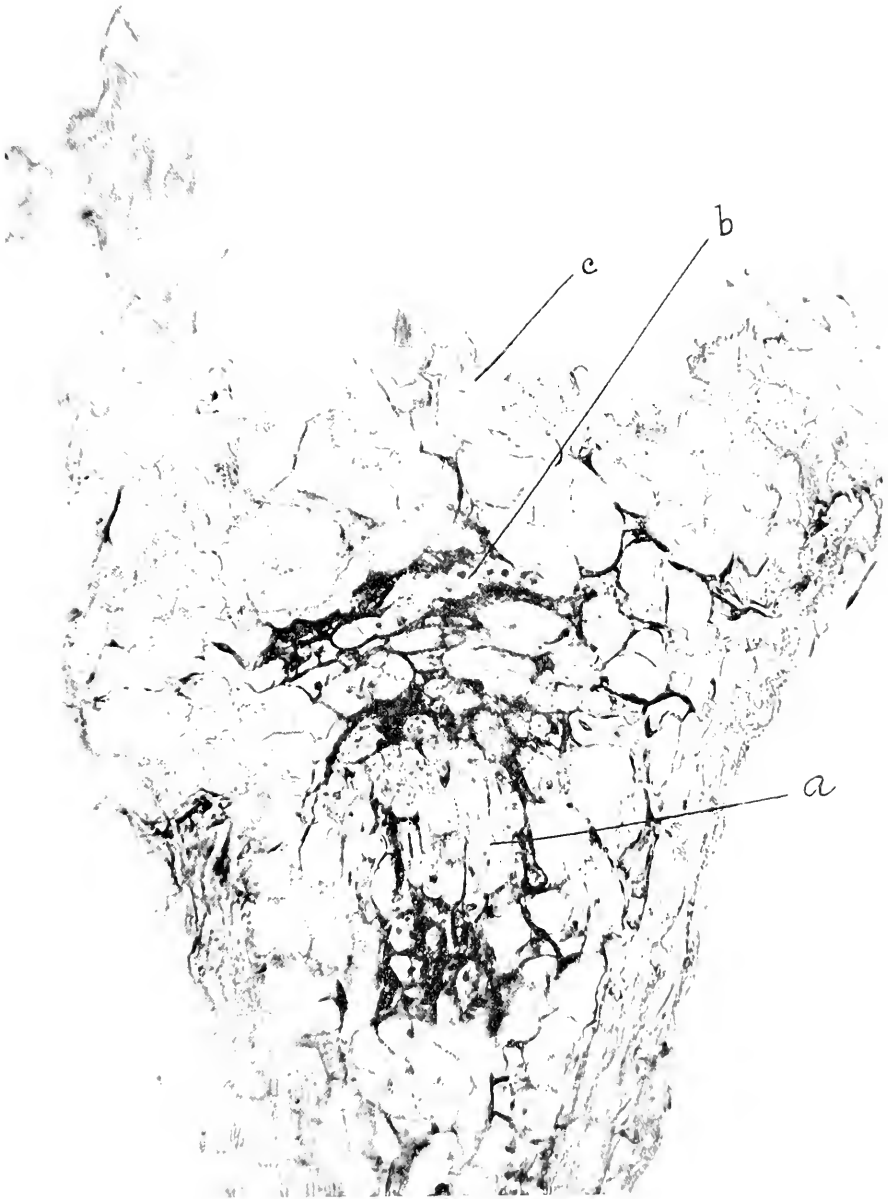


PLATE 38

Fig. 1. Teratological tissue in F₁ hybrid *Crepis capillaris* × *tectorum*. Z 5. Cross-section of root just below ground level. × 530.

a. A vegetative cell dividing (metaphase). This cell is completely surrounded by fully differentiated cells.

b. Very much crumpled and distorted cells of outer wall of seedling.

c. Black areas showing decomposition of cells.

Fig. 2. Chromosomes of *Crepis capillaris*. Polar view showing two *J*'s and four more or less rodlike. × 1500.

Fig. 3. Chromosomes of F₁ hybrid *C. capillaris* × *C. tectorum* (Z 5) showing two *J*'s, one *V*, and rodlike ones. × 1500.

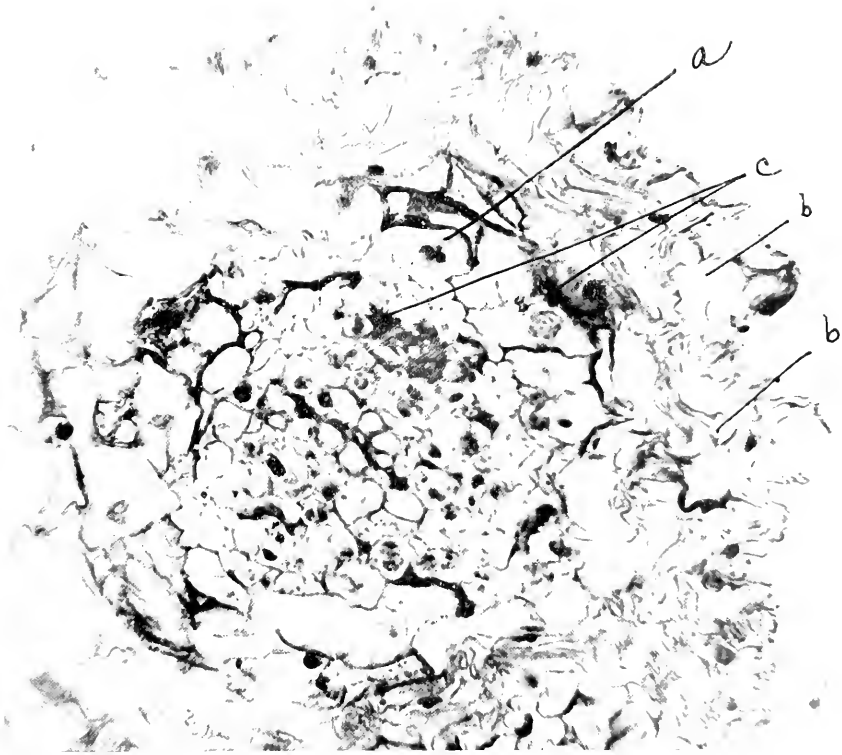


Fig. 1

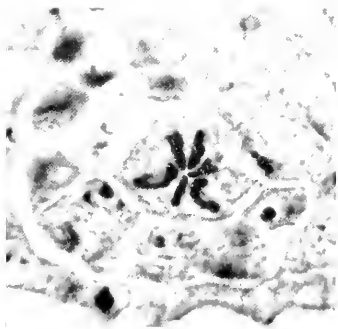


Fig. 2

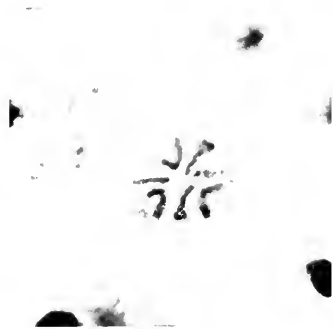


Fig. 3

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November 23, 1920

INBREEDING AND CROSSBREEDING IN
CREPIS CAPILLARIS (L.) WALLR.

BY

JULIUS L. COLLINS

UNIVERSITY OF CALIFORNIA PRESS
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November 23, 1920

INBREEDING AND CROSSBREEDING IN
CREPIS CAPILLARIS (L.) WALLR.

BY
JULIUS L. COLLINS

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INTRODUCTION AND BRIEF DISCUSSION OF
INBREEDING EFFECTS

It is well established that continued inbreeding within a strain or race may produce results harmful to individuals of that race. It is only in modern times, however, that a consistent explanation of the causes of such results has been made. This explanation of the problem has come through carefully planned and executed experiments upon plants and animals and through a recognition of the Mendelian laws of heredity. The most extensive and comprehensive of these investigations is that with maize, started by East¹ in 1905 and now being carried on by Jones, at the Connecticut Agricultural Experiment Station.

Inbreeding is now considered and used as a method by which the hereditary constitution of the germ-plasm can be made evident. Inbreeding, as such, produces no evil results. The abnormal forms that sometimes appear in inbred strains show up because the recessive genes conditioning such forms are present in the germ-plasm. If no such genes are present, no amount of inbreeding can produce them.

The fact that inbreeding produces abnormal forms and reduction of vigor in some species and not in others is due to a condition of the germ-plasm. For example, no such results attend inbreeding in self-fertilized crops like wheat and barley because in them self-fertilization is the normal method of reproduction and such plants are homozygous for all their genes, all the abnormal and weak plants

¹ East, E. M., and Jones, D. F., *Inbreeding and Outbreeding*, pp. 1-285, 46 illus., Philadelphia, Lippincott. 1919.

having long ago been segregated out of the race and having perished in the competition with their more hardy and vigorous sibs.

Maize is a naturally cross-fertilized species, and heterozygosity is therefore the general condition of the germinal material instead of homozygosity, as is the case in self-fertilized species. In this heterozygous condition the genes of recessive harmful characters may be carried along in the germ-plasm under the protection of desirable dominant characters and appear only when the latter are absent. Inbreeding furnishes conditions favorable to the accumulation of these recessive genes in the germ-plasm and for the appearance of the recessive characters in some of the individuals.

The increase in size and vigor observed in the progeny when two different inbred strains, or inbred strains and unrelated non-inbred strains, are crossed is due to the establishment of a heterozygous germ-plasm containing more dominant factors influencing size and vigor than were present in either of the parents. Linkage of such genes to recessive or to dominant genes which influence vigor and size adversely prevents the production of homozygous dominant races. For this reason the vigor noticed in the F_1 is less marked in the F_2 and subsequent generations where segregation and recombination have taken place.

Most of the knowledge of the effects of continued inbreeding and the results obtained from crossing inbred strains have come from experiments on plants and animals under domestication. Such species have been the subject of conscious selection for particular types, which often preserves in the species characters desirable from an agricultural point of view, but so detrimental that the race could not exist except under the conditions of domestication. It has been questioned whether the germinal material of such races is comparable to that of wild species in which natural selection may have largely eliminated from the germ-plasm genes which produce characters detrimental to the natural existence of the species. In view of this possibility the question has arisen as to whether or not the results of continued inbreeding would be the same if wild species, in place of domesticated ones, were the subject of such experimentation. It is in this connection that this report on inbreeding in *Crepis* is of interest.

MATERIAL AND METHODS

Crepis capillaris is a species belonging to the chicory tribe of the Compositae. The species is a native weed of European and North African countries, and has been introduced into both North and South America, where it grows in limited localities as a common weed. It is either annual or biennial. The flowers are all perfect and both cross and self-fertilization take place under natural conditions. In nature it is quite variable in a number of ways according to the environmental conditions in which it grows, but our breeding experiments show that, when grown continuously under the same conditions, constant forms are produced in successive generations. No records have been found of its subjection to extensive artificial selection and it is therefore, in the true meaning of the word, a wild species.

In order to reduce the effect of variation in the environmental factors of soil, light, temperature, moisture, and space to the minimum care was taken to have the soil homogeneous, to have the same size and kind of pots, and to grow successive generations of plants in the same portion of the greenhouse as their parents had occupied. This last item was varied in the last generation (1920) to the extent of placing both inbred and hybrid cultures on a bench on the opposite side of the greenhouse from the side where the parent cultures grew. Inbred and hybrid cultures have been grown side by side. The arrangement in plate 39, figure 1, and plate 40, figure 1, is that in which the plants grew on the bench. Some of the inbred plants and some of the crossbred plants were grown in both four and six-inch pots. This did not alter the size and growth relations in any way.

Crossing was accomplished in cultures 115 and 129 by emasculation² of the plants intended to be female parents, while in II-10 the water² method of depollination was used.

² To be described in detail in another paper.

INBREEDING AND CROSSBREEDING EXPERIMENTS

Cultures of *Crepis* were first grown to study and to isolate certain character variations which had been observed. Forced inbreeding was resorted to as the quickest means of reaching the desired end. After two generations of inbreeding it was noticed that the plants were much smaller and less hardy than at first, notwithstanding the fact that cultural methods had not varied to any marked extent. Experiments were then planned and executed with these cultures to demonstrate the effects of continued inbreeding and subsequent crossing in a wild species, and the results obtained form the body of this report.

In Table 1 are given the pedigrees of the cultures in which inbreeding was continued. Cultures 20.113, 20.114, and 20.128 have identical ancestors previous to their parents, which were sibs. In the second generation of inbreeding, sibs of culture e2 were crossed because the strain showed such a high degree of self-sterility that it was feared that not enough viable seed could be secured to maintain the strain. By crossing sibs, which, however, were very similar in all respects, a few viable seeds were secured. Self-fertilized seeds of e32P₆ and of e32P₁₈ were also secured and their cultures were in all measurable respects no less vigorous than the progeny of crossed sibs of culture e32. Plate 41, figure 1, shows culture 113, derived from crossing sibs, and culture 114, derived from selfing one of the sibs used in the cross. Thus for inbreeding purposes the culture e2 had reached an almost homozygous condition in the third generation, since in no case have appreciable changes been noticed in the fourth generation.

Culture e28 also seemed to reach its maximum reduction of vigor and size in the third generation of inbreeding. No crossbred plants from this inbred strain have yet been grown. In contrast, H-10 (pl. 41, fig. 2), resulting from crossing sibs in the two previous generations, shows but little reduction in vigor or size, indicating that it is either still heterozygous genetically or is not affected by inbreeding to the same degree as e28. The latter seems to the writer more probable, inasmuch as the entire culture of H-10 was fairly uniform, thus indicating a large degree of homozygosity.

Cultures 17.192 and Z9 used in the crossbreeding experiments were chosen because they could have no immediate genetic relation

to the inbred cultures. Seed secured from wild plants in Berkeley, California, was used in 1916 to start the culture 17.192. Cambridge (Quy Fen), England, is the source of culture Z9. The latter were slightly more vigorous than the Berkeley plants. Culture 17.178 was grown from seeds from wild plants found growing near Eureka, Humboldt County, California.

Culture e33, which was used as one of the parents of crossbred culture 129, is a reciprocal of e32, and similar in all respects.

Pedigrees of the plants used in these experiments are shown in the accompanying tables. In Table 1 two systems of symbols are used to designate cultures. In the parent stock and in the first and fourth generations the annual-notebook-page-number system of Shull is used. In the second and third generations individual cultures are designated by key letters combined with numbers. In both tables individual plant numbers are designated by P with a subscript. In Table 2 the same systems are used together with special key letters (H and Z) to designate certain cultures.

TABLE 1—SHOWING PEDIGREES OF PLANTS USED IN THE INBREEDING EXPERIMENT

Parent Stock	Generations of Breeding			
	First	Second	Third	Fourth
17.178P ₆₀	18.1SP ₅	e2P _{2×16}	e32P _{6×12}	20.113
17.178P ₆₀	18.1SP ₅	e2P _{2×16}	e32P ₆	20.114
17.178P ₆₀	18.1SP ₅	e2P _{2×16}	e32P ₁₈	20.128
17.178P ₁₂	18.31P ₉	e8P ₆₂	e2S	

TABLE 2—PEDIGREES OF CROSSBRED PLANTS

	17.192P _{7×4}	—204P _{46×27}	—H10	
	17.192P _{7×4}	—204P _{27×6}	—H6P ₈	} —20.129
			×	
17.178P ₆₀	—18.1SP ₅	—e2P _{16×2}	—e33P ₆	
			×	} —20.115
17.178P ₆₀	—18.1SP ₅	—e2P _{2×16}	—e32P ₁₅	
			Z9P ₆	

DISCUSSION OF RESULTS

One would expect that the germ-plasm of an old wild species had been largely purified of the genes which cause the production of abnormal and harmful characters by the elimination of weak forms through natural selection, but our experience with *Crepis* demonstrates that such is not the case in a race partially cross-pollinated. The germinal material of *Crepis capillaris* is maintained in a heterozygous condition by natural cross-pollination, as is the case in the cultivated species of maize. This is shown first by the marked reduction in the size of the plants and their slower rate of development, and secondly by the fact that we have isolated, by inbreeding and selection, constant breeding forms which differ in the characters for which selected.

The maximum amount of the effects of inbreeding appears to occur in the second and third generations. Forms have been observed in inbred cultures which have not been observed in wild colonies; no doubt a more extended observation would show that they do occur, though rarely. Pollen sterility is one of the results of inbreeding and one plant has appeared in a third generation culture which produced almost no pollen. In the culture produced by growing seed of wild plants which themselves grew in New Zealand we have also found one plant (N. Z. P., 1920) which produces no pollen at all; other plants of this culture appear normal in pollen production. This is evidence that this character may also appear in wild plants.

Strains of fasciated plants have been isolated in *Crepis tectorum* which are so weak that it is only by starting a large number that we can get a few to live long enough to produce seed, yet the plants in the heterozygous condition seem to be in no way affected.

Most of the plants were grown in four-inch clay greenhouse pots. In order to determine whether this limiting of the root space would in any way accentuate the dwarfishness of the inbred plants, part of inbred strain No. 128 and part of hybrid culture No. 129 were grown in both four-inch and six-inch clay pots and placed in adjacent rows on the bench. Plate 40 showing plants in six-inch pots and plate 39 showing similar cultures in four-inch pots answer this question in a very definite manner.

The results of inbreeding in *Crepis* support the statement of East and Jones³ that in naturally cross-fertilized organisms the immediate results of inbreeding are most emphatically injurious, but it must be considered as an exception to their statement that "wild types, in general, might not present such an appearance of injury under inbreeding as shown by cultivated species." Maize is characterized by the occurrence of both cross and self-fertilization, and when this condition exists in wild species we may expect such species to behave like maize when subjected to forced inbreeding.

SUMMARY

Inbreeding in a naturally cross-fertilized wild plant, *Crepis capillaris*, causes conditions in many ways similar to the conditions produced by inbreeding in maize.

The maximum reduction appears to be reached in the third and fourth generations.

Crossing inbred strains with non-inbred strains produces vigorous, rapidly growing F_1 plants.

Inbred plants, when compared with crossbred plants, show a slower rate of development during the entire period of growth.

Some of the inbred strains showed pollen sterility by a reduction in the number of mature pollen grains formed.

Increased size of pots and quantity of soil did not affect the relationship of vigor and of growth.

The results of the experiments on *Crepis* indicate that the results of inbreeding maize as reported by East and Jones⁴ and others are in no way peculiar to that species, but may be found to hold for other species, either domesticated or wild, when similar conditions affecting sexual reproduction obtain.

³ *Op. cit.*

⁴ *Op. cit.*

PLATE 39

Crepis capillaris

Fig. 1. At right and left are plants representing the fourth generation of inbreeding in two related strains.

The central plant is the result of crossing an inbred plant of the third generation with a totally unrelated non-inbred strain of *Crepis*.

Fig. 2. The same three plants as shown in figure 1, photographed about six weeks later, showing the precociousness of the hybrid (115) when compared with the inbred plants.

Plants growing in four-inch pots.

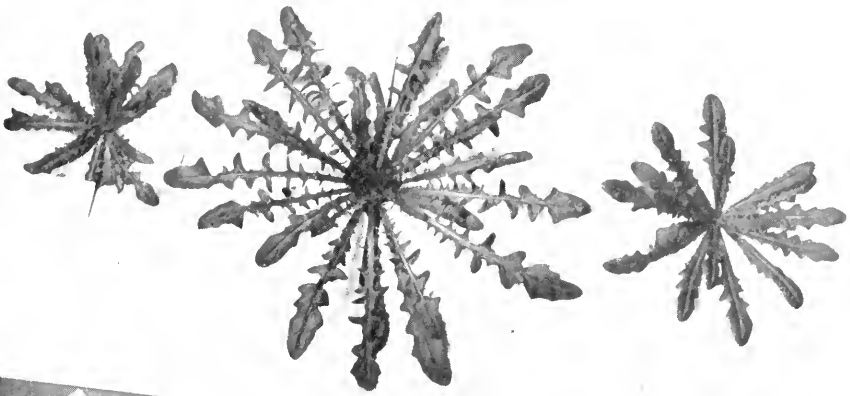


Fig. 1

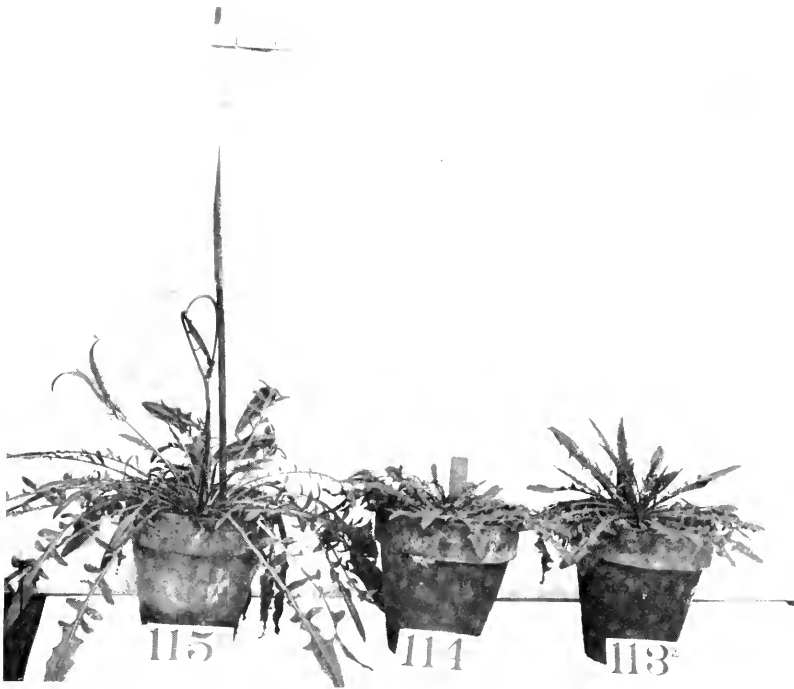


Fig. 2

PLATE 40

Crepis capillaris

Fig. 1. 20.129/4. Hybrid secured by crossing an inbred plant with a non-related non-inbred plant.

20.128/10. Inbred plant of the fourth generation; progeny of the inbred parent of the hybrid plant 129P4.

Fig. 2. The same two plants photographed about six weeks later showing marked vigor of the hybrid.

Growing in six-inch pots.

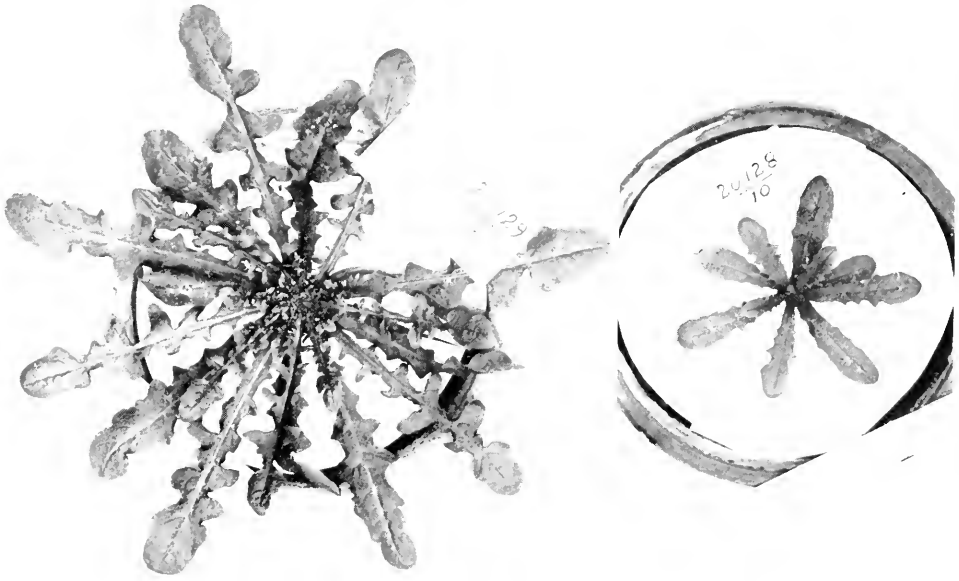


Fig. 1

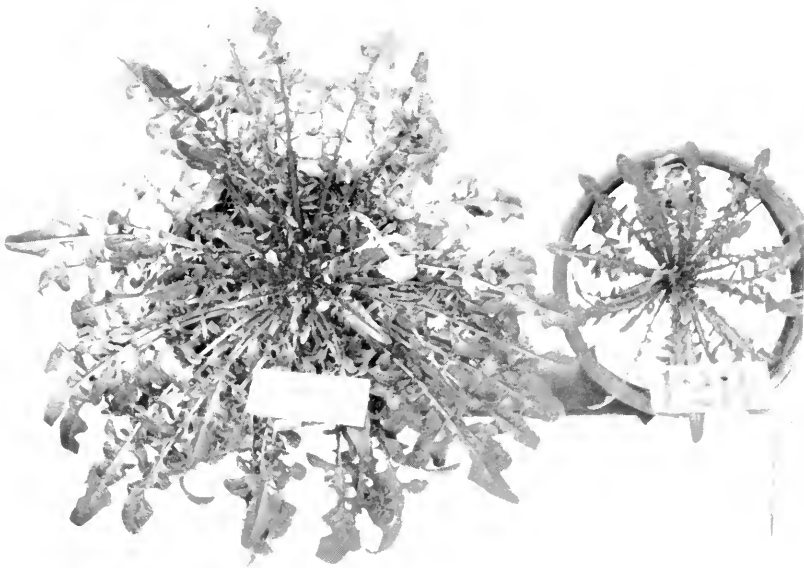


Fig. 2



PLATE 41

Crepis capillaris

Fig. 1. Cultures 113 and 114 are inbred plants of the fourth generation. Culture 115, F₁ hybrid plants of the same age as the cultures 113 and 114.

Fig. 2. e28/25 and e28/21, inbred plants. H10/11. A non-related plant produced by continual crossing of sibs. Growing in four-inch pots.

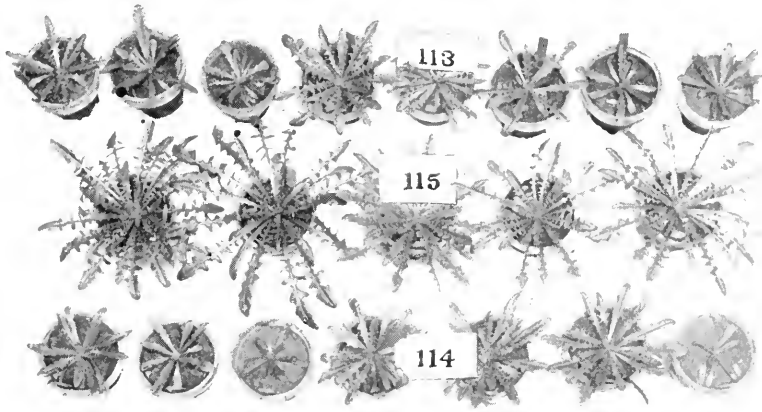


Fig. 1



Fig. 2

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June 8, 1923

INHERITANCE OF SOME MORPHOLOGICAL
CHARACTERS IN CREPIS CAPILLARIS

BY

VENKATA RAU

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY, CALIFORNIA

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INHERITANCE OF SOME MORPHOLOGICAL CHARACTERS IN *CREPIS CAPILLARIS**

BY
VENKATA RAU

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INTRODUCTION

Geneticists studying the inheritance of characters in plants have been following with interest the monumental investigations on *Drosophila* by Morgan and others, with especial attention to their studies on the inheritance of both qualitative and quantitative characters. The present paper reports the result of an investigation on the inheritance of some quantitative characters in a wild plant, *Crepis capillaris* (L) Wallr. The studies included characters in leaves and flowers, and it will be shown that the inheritance of these characters is similar to the inheritance of quantitative characters in other organisms.

*Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of California.

OBJECTS AND AIMS

The genus *Crepis*, comprising over 150 species, belongs to the tribe Cichorieae of the natural order Compositae, and is closely related to the genus *Hieracium*. The species, *C. capillaris*, so far as known, has not been brought under cultivation, but grows as a wild plant in widely separated parts of the world. This species can be easily propagated and the plants are self-fertile so that investigations may be carried on with inbred strains. Furthermore, the F_1 and F_2 generations from varietal crosses are fertile when crossed *inter se*, and the species has a very low number of chromosomes. Hence as Babcock (1920) pointed out, the advantages of the genus for genetic investigation are many. Previous to that, some work had been done on the cytological side, notably by Rosenberg (1909-1918), who determined the number of chromosomes, Beer (1912), Miss Digby (1914) and de Smet (1914). De Smet has given excellent illustrations of the various stages of nuclear division. Other species of *Crepis* have been studied by Rosenberg (1909-1918) and Juel (1905); interspecific crosses between *C. capillaris* and *C. tectorum* have been reported by Babcock and Collins (1920). The achenes of *C. capillaris* germinate easily after a short period of rest and a very large percentage is viable. The plant first develops a rosette and finally the central axis elongates and terminates in an inflorescence; but under unfavorable conditions it may remain indefinitely in the rosette stage. The plant is strictly annual, however, and dies after once flowering. Plate 43 illustrates typical plants when the inflorescence has developed and growth has practically ended.

The present investigation has to do specifically with differences in the length of the radical leaves, in the number of lobes on the radical leaves, and in the diameter of the flower heads. The aim was to determine whether these differences were inherited and to locate the factors responsible for the genetic variations as distinct from modifications due to the environment. In the case of the inheritance of morphological characters in the leaf, the action of the environment had to be taken into consideration, and in the case of the flowers, the action of the environment in addition to the age of the plant and the position of the capitulum upon the plant had to be evaluated before the true genetic variations could be determined. The work has been carried on

partly in the greenhouse and partly in the field and the results have been found so consistent that the data have been combined. The investigations herein reported were started in the fall of 1920 and were carried on by the writer until July, 1922, but a great deal of preliminary purification of material had been done before the material was turned over to me.

The work was undertaken at the suggestion of Professor E. B. Babcock, head of the Division of Genetics, University of California, to whom my best thanks are due. My thanks are also due to Dr. R. E. Clausen and Mr. J. L. Collins, of the Division of Genetics, for especially valuable help and suggestions during the progress of the investigations.

MATERIAL AND METHODS

The detailed work has been done on three inbred families. The achenes were always germinated in seed pans in which the soil had been sterilized, or which had been filled with soil near which no *Crepis* plants had been grown within the last few years. The achenes were lightly covered with soil and watered. The germination was fairly rapid and the seedlings were ready for transplantation in about four weeks from the date of sowing. They were transferred either to small cardboard boxes about two inches square and planted out in the field or to 4-inch or 6-inch pots directly. The size of the pot had very little influence on the early development of the plant although, so far as general vigor was concerned, the plants in the 6-inch pots gave better results.

In measuring the length of the leaves and determining their lobe number, the plants were allowed to develop as far as possible in the rosette stage and data were secured before the central axis appeared with the formation of the cauline leaves. The length of the leaf was measured on a centimeter scale and the number of lobes counted on one side of the leaf, usually the left side. Every lobe which was supplied with a distinct vein was given a unit rank and in these calculations all scurs at the base of the leaf and the secondary lobes attached to the main ones were not considered. Five leaves were indiscriminately chosen and counts made upon them.

The capitula were measured on the centimeter scale when they were fully open. Flower heads in *Crepis* open centripetally, and a

flower head was considered fully open when all the disc florets had opened and the stigmas were projecting. This stage is usually maintained for two or three days. Then the capitula widen and spread out, and measurements taken at this stage always give results which are about 3 mm. more than the actual diameter when the heads are fully open. Moreover the flowers open at about 9 a.m. on bright days and remain open till after 3 p.m. if the day is not hot. But on dull and cloudy days they open about 10 a.m. or later, and occasionally they fail to open altogether. The 25 flowers first formed were measured in every case and their individual measurements noted. Inflorescence in *Crepis* closely follows the type described by Gleason (1919) for *Vernonia mussurica*. The main axis is the first to give off flowers, and the few branches at the top are more or less leafless. The flowers form a more or less flattened corymb at the top. The lower nodes bear shorter and frequently less developed lateral branches which usually appear so late in the season that none of the heads, or only a part of them, open their flowers and set seed before the plant has exhausted itself and dies down. In *Vernonia* three types of variations were investigated: (1) a variation between the heads of each cyme, possibly correlated with their position whether terminal or inferior; (2) a variation between different floriferous branches of the same plant possibly correlated with the amount of available nourishment; (3) a general variation between different individuals, possibly correlated with the size and vigor of the plant and therefore indirectly with the habitat. Gleason finds that within a single cyme of from two to six heads the terminal head is the largest. In larger cymes, some of the secondary terminal heads are frequently larger than the primary terminal head, the number of flowers is greatest for the terminal head of each cyme, but it is relatively constant for each individual plant. Two sets of factors, which may be environmental, or hereditary, or both, are involved. One determines the number of heads produced and the other the average number of flowers in each head. These act upon the plant independently and thus give four classes: many large heads, many small heads, few large heads, and few small heads. This investigator based his measurements and conclusion on 25 flowers. Goodspeed and Clausen (1915) estimate 25 as the minimum number on which to base any calculations for flower size. Goodspeed and Clausen (1918) have described a mechanical apparatus by which measurement of flowers is made. East uses only a millimeter scale; I have followed East in this work.

With regard to the method of cross-pollinating the plants, both the methods suggested by Babcock and Collins (1920) were tried, and depollination with a water jet has given results as good as emasculation, although the latter method was employed in all cases of critical investigation. The flowers were enclosed in translucent paper bags to prevent insect pollination and the achenes gathered before they were over-ripe and dropped to the ground or were taken off by the wind. It is fairly easy to decide whether a cross-pollination has been successful or not because the involucre assumes an ovoid form in the successful crosses, whereas it remains more or less oblong in the unsuccessful ones. The achenes, moreover, are plump and the ribs marked, the seed coat itself being distinctly colored as compared with that of the unfertilized achenes.

INHERITANCE OF LENGTH OF LEAF

In *Crepis capillaris* the first true leaves are small (about twice the size of the cotyledons), and there is a continuous increase in leaf size until the rosette is formed. Plate 42 shows stages of growth of the leaves including the mature rosette when they are ready for measuring. Even in the early stages the plants show different habits of growth, some growing erect and others spreading horizontally. In one family especially (20.6) there is a tendency for the leaf margins to curl downward, thus rendering measurement difficult (plate 42, fig. 4). In the earlier work, the leaves were clipped off with a pair of fine scissors close to the stem and measured on a centimeter ruler. But later on it was thought that injuring the plants thus might affect the result, and the leaves were kept intact on the plant while the ruler was thrust in as close to the stem as possible. Five mature leaves were measured at random and the average of the readings has been taken to represent the mean length of leaf in the plant. In table 1 it will be seen that the length of leaf fluctuates widely from the mean as compared with the breadth. The variation in length was 12.6 to 23.0 cm. in family 20.1, 11.8 to 18.4 cm. in family 20.6, 15.8 to 30.7 cm. in family 20.11 and from 24.0 to 40.1 cm. in family 20.13. Crosses were made between the 20.1 family with a range from 13 to 23 cm., and family 20.13 with a spread of 21.0 to 40.1 cm. with a view to studying the way in which the factors for length segregated. Table 2 gives the usual biometrical data for the various families studied. This table indicates that the factors for length show segregation in F_2 , but owing to the fact that the environment plays such a great part in

determining the length, it is difficult to estimate the number of factors involved. (See Hayes, 1912, p. 34.) Figure 1 shows the length of leaves typical of the parent races, and typical leaves from the F_1 population. Figure 2 shows typical leaves from plants of the F_2 generation. The drawings have been made from actual prints of leaves on photographic paper and reduced equally in reproduction.

TABLE 1
SHOWING MEASUREMENTS OF LENGTH AND WIDTH OF LEAVES

20.1		20.6		20.11		20.13	
Length cm.	Width cm.	Length cm.	Width cm.	Length cm.	Width cm.	Length cm.	Width cm.
22.2	3.9	18.1	4.5	15.8	3.1	29.4	4.0
17.3	3.6	15.5	4.3	23.3	4.4	34.0	5.0
17.2	3.5	14.6	3.7	25.5	6.8	35.0	6.0
15.0	2.5	16.3	3.5	30.7	6.8	30.0	3.6
18.4	2.8	11.8	2.0	20.0	4.5	40.1	5.8
15.5	3.4	16.1	3.7	29.5	7.3	26.1	5.1
15.6	3.4	16.0	3.7	32.2	5.6	37.7	6.5
15.6	3.4	14.7	3.2	28.6	6.0	26.0	3.5
20.0	3.4	18.4	4.5	18.5	5.0	24.0	3.0
23.0	4.4	13.2	2.5	28.6	6.0	28.0	6.0
19.8	2.9	14.9	3.6			33.0	4.5
12.6	2.0	15.8	3.1			34.0	6.0
						24.0	3.5
						34.0	5.6
						29.0	5.0
						31.0	4.0
						31.5	3.8
						23.0	3.0
						31.0	5.0
						21.0	2.5
						21.6	3.0
						29.5	4.3
Total:							
212.2	35.8	185.4	42.3	252.7	55.5	652.9	98.7
Average:							
17.7	3.0	15.45	3.5	25.3	5.5	29.7	4.5

It should be stated that the plants of the F_2 population were grown in 4-inch pots while those of the parent races and F_1 population were in 6-inch pots. However, the F_2 plants were all grown under uniform conditions so that the evidence of segregation in both leaf length and number of lobes may be referred to genetic differences among the F_2 plants.

INHERITANCE OF THE NUMBER OF LOBES

The problem of the number of lobes on the leaves resolves itself into four distinct subheads. The first of these involves the question whether the leaf shall be considered lobed at all. There are families in which the lobing, if present, is so shallow that the leaves would be described as entire or merely dentate. This type is designated as

TABLE 2
SHOWING THE RESULTS OF CROSSING FOR INHERITANCE OF LEAF LENGTH

Nature of Cross	Generation	Mean	Stand. deviation	Coef. of Var.
20.1 x 20.13	P ₁	17.9 ± .588	2.89 ± .468	16.1
	P ₁	29.7 ± .699	4.97 ± .495	16.7
	F ₁	29.0 ± .282	2.33 ± .188	8.0
	F ₂	14.9 ± .137	5.28 ± .097	35.4

Applying Castle's formula

$$n = \frac{(29.7 - 17.9)^2}{8(5.28^2 - 2.33^2)} = \frac{139.24}{179.2}$$

Factors responsible for length = 1 factor.

This result is very improbable, but the results can be interpreted on a modified dihybrid ratio of 9:6:1 where the two single homozygous genotypes give identical effects. On this ratio and from a study of the data, the result may be stated thus:

- A B = 9, leaf length from 6 - 18 cm.
- A b = 3, leaf length from 19 - 25 cm.
- a B = 3, leaf length from 19 - 25 cm.
- a b = 1, leaf length from 26 - 34 cm.

Where factors A and B stand for two independent factors in the absence of both of which the double recessive a b is obtained:

- Observed numbers: 491 : 158 : 27
- Calculated numbers: 378 : 252 : 42.

simplex in the accompanying account. There is another type where the lobes are distinct and simple and look like the steps on a ladder. This is designated as the sealaris type. A third type has a complex type of lobes where the sealaris type of lobing is surmounted by smaller secondary lobules or wings. The second subhead refers to the incision or depth of lobing. In the families studied the lobing extended halfway from the margin to the mid-rib or completely to the mid-rib. The third subhead concerns number of lobes on the leaf and the fourth refers to the character which is shown when the secondary lobules instead of remaining attached to the main lobes are

separated and form independent lobes attached to the mid-ribs. The first of these is the major character because, without a tendency to form the lobes, the rest of the factors could not express themselves. But the remaining three subheads behave as separate groups of factors, the depth of incision having an independent action on the leaf as do the other two characters mentioned above. One thing, however, was clear from the studies made, and that was the complex way in which

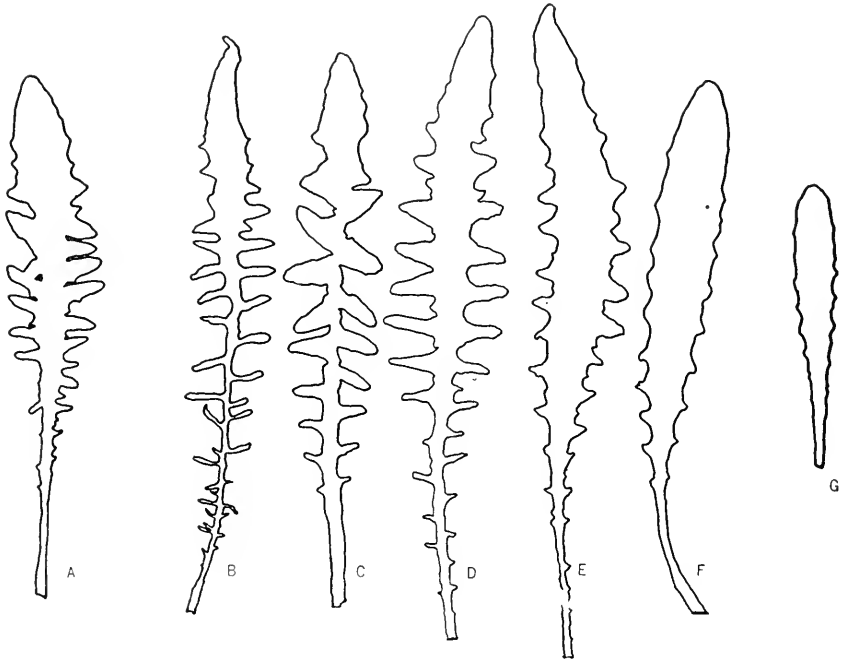


Fig. 1a. A typical leaf of the race with long leaves and many lobes. *g*. A typical leaf of the race with short leaves and few lobes. *b-f*. Typical leaves from different plants of the F_1 generation. *c*. $\times \frac{1}{3}$.

each of these characters was inherited. That these groups of characters are inherited in a Mendelian fashion cannot be doubted, but the work has not advanced enough to estimate with certainty the number of factors involved in these cases, except in the number of lobes, which has been more extensively studied.

The same families that furnished material for studying the inheritance of length have been used for studying the lobe numbers. Table 3 shows the lobe numbers of the various families handled in this work. The same illustrations, figures 1 and 2, show the nature of lobing and the number of lobes.

TABLE 3

SHOWING THE RESULTS OF CROSSING FOR INHERITANCE OF NUMBER OF LOBES

Nature of Cross	Generation	Mean	Stand. deviation	Coef. of Var.
20.1 x 20.13	P ₁	8.9 ± .352	1.73 ± .248	19.4
	P ₁	11.3 ± .171	1.21 ± .134	10.7
	F ₁	11.17 ± .156	1.37 ± .110	12.2
	F ₂	8.1 ± .087	3.36 ± .061	41.5

Applying Castle's formula the number of factors would be

$$\frac{(11.3 - 8.9)^2}{8(3.36^2 - 1.37^2)} = \frac{5.76}{75.2}$$

an obvious impossibility.

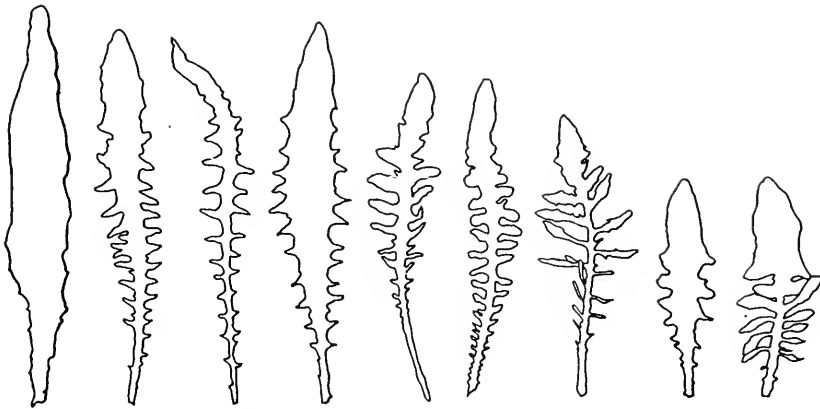


Fig. 2.—Typical leaves from different plants of the F₂ generation. c. x 1/3.

The data can be interpreted on a four factor hypothesis where each factor in a homozygous condition contributes two lobes and, in a heterozygous condition, one lobe. On this hypothesis the genotypic formula would be,

- a a b b c c d d 5
- a a b B c c d d 6
- a a B B c c d d 7
- a A B B c c d d 8
- A A B B c c d d 9
- A A B B C c d d 10
- A A B B C C d d 11
- A A B B C C D d 12
- A A B B C C D D 13

and the data on this hypothesis would give a curve which simulates the normal curve of error with the mode at 8.

From the data presented in table 3, it is fair to conclude that there is segregation with respect to mean lobe number in F_2 . Both the F_1 and F_2 are intermediate between the two grandparent types and in the latter there is no transgressive segregation on the side of the higher number of lobes. The number of lobes ranges from 6 to 13 in the F_2 family 21.141 and arranging the plants in class groups their distribution is as follows, the mean being at 9.

6	31
7	42
8	54
9	35
10	47
11	37
12	9
13	1
<hr style="width: 20%; margin: 0 auto;"/>	
256	

This tabulation shows that the inheritance of lobe number is complicated; and, while more of the plants show the lobe number of the lower numbered parent, the majority of them are intermediate as required by the hypothesis of multiple factors. The same remarks apply to the other F_2 populations studied, and there must be at least four factors responsible for number of lobes in the leaves.

The length of the leaf has little or no influence upon the number of lobes in the leaves. The accompanying correlation chart, table 4,

TABLE 4
CORRELATION TABLE FOR NUMBER OF LOBES (x) AND LENGTH OF LEAF IN CM. (y)
FAMILY 21.140

	4	5	6	7	Σy
8-11	10	4	14
11-14	1	15	25	41
14-17	11	17	1	29
17-20	8	46	6	60
20-23	9	54	4	67
23-26	9	38	1	48
26-29	4	8	0	12
Σx	1	66	192	12	

$$r_{xy} = 0.2302 \pm 0.0388$$

constructed for family 21.140, shows that the correlation between the two is very low. For purposes of calculation, length of lobe is expressed in round numbers of centimeters, the fraction being treated as one when more than half and ignored when less than that amount.

The absence of influence of length of leaf on number of lobes is also illustrated by a comparison of the leaf outlines which show practically the same number of lobes on leaves of different lengths and in other cases different numbers of lobes on leaves of practically the same length. From an extended study of the data as well as from observations in the field and green house on various races of *Crepis capillaris*, I am led to conclude that number of lobes is a definitely heritable character and is not influenced by length of leaf, by soil or by any other environmental conditions under which the plant is grown.

INHERITANCE OF SIZE OF CAPITULUM

Goodspeed and Clausen (1915) have determined a number of factors which influence flower size in *Nicotiana*. Under the heading, "age of plant," they have considered the difference in size of flowers borne early in the season as compared with those borne late in the season on the same plants as well as the difference in size of flowers during the first blooming season of the plant compared with that of flowers produced the next year and on the same plants cut back and sprouting from the roots. Under the heading "age of flower," they include, first, a consideration of the difference in the size of flowers borne on the terminal inflorescences first coming out of the stem and those borne at the same time on laterals and seconds, and (2), the influence of age on the individual flower by comparing measurements of flowers fully opened before and after shedding pollen. Other factors such as influence of removal of flowers and developing seed capsules, the behavior of cuttings under various conditions, and the influence of soil fertility were also studied. They find that the flowers produced later in the season have usually been of smaller size. By removing all flowers as fast as they are produced, they find it possible to keep the flower size nearly equal to that of the first flowers produced and were able in some cases to double the length of a plant's life. During the period which elapses from the time a flower is fully opened to the time when pollen is shed, there is a considerable increase in corolla spread, and associated with it, little or no increase in corolla

length. Soil also had a great influence in their experiments in determining the size of the flowers. "The conclusion seems irresistible that flower size in *Nicotiana* is not so constant as it has been assumed to be, but that it is affected by a number of conditions and that at least some of these may not affect the length and spread in the same manner."

INFLUENCE OF AGE OF PLANT

In *Crepis capillaris*, the 25 capitula first formed are usually very uniform and show a very narrow range of variation. The terminal flower is usually the largest, although the next two flowers below it are of the same size in many instances; but usually there is a significant difference of 1 mm. when a large number of flowers are measured. The flowers were pulled off and measured in every instance, which eliminated to a large extent the possibility of the flowers' growing slightly smaller. As a rule the 25 flowers required were measured in about a week's time, although the plant normally continues to flower for about four to five weeks. Flowers measured at the end of a season are about 15 to 20 per cent smaller than those measured at the beginning and, owing to the setting of seed and senility of the plant, all the buds formed do not open. In an experiment which was carried on to measure the entire lot of flowers that were produced on 6 plants of a strain, the plants started flowering on the tenth of February and continued till the end of April. Comparing the early flowers with those formed later, the size of the latter is smaller. But this reduction is not so great as in the case of plants from which no flowers are removed. Two things can be noted, however, in the flowers formed later. The number of flower heads that open on any given day is less than before and the number of florets per head is significantly smaller, the capitulum showing a more open center. The actual size of the floret is not perceptibly reduced and this accounts for the fact that the size of the flowers remains fairly constant. Another character that can be seen in the flower heads formed later is the slender elongated stalks on which they are borne as compared with the robust stalks of the earlier formed flower heads, while in many cases the internodes between the flower stalks are longer in the later formed flowers.

the inflorescence and furnishes the measurements of the diameters of individual capitula of a single plant. Comparing the individual cymose clusters, the terminal cluster has the largest central flower: closely followed by the next few lower clusters. As the measurements are followed farther down, the central capitulum becomes slightly smaller. The lateral capitula are generally smaller than the central capitulum in each cluster, but at times they may attain to the same size, especially in the uppermost cymes. Very rarely they are larger than the central capitulum of the cyme of which they are laterals. The central capitula of the lower cymes may be larger than the lateral capitula of the upper cymes. In comparing flower heads as to size, however, the facts that all the capitula do not ripen at the same time and that the age of the plant is a factor causing variation should be kept in mind. Moreover, in this group of measurements, the flowers were pulled off for measuring, and this has a tendency to keep the inflorescence active for a longer time and to maintain the flower size, as has been noted by Goodspeed and Clausen. The facts as to variation of size in the flowers, due to the age and position of the flower, may be summarized by saying that, in plants allowed to flower normally, the terminal flower head is usually the largest, closely followed by the second and third flower heads, after which the size becomes slightly smaller. The relative size of the flowers on the lower branches is similar, but the terminal flowers on the lower branches are smaller than the terminal flower of the whole plant or than those terminal flowers which arise from branches in the axils of the uppermost leaves.

ENVIRONMENTAL FACTORS

Light.—With regard to the effect of light on the flowering of plants, some interesting results have been obtained. Klebs (1918) in his work on *Sempervivum* divided the process of flower formation into three distinct stages: (1) production of the condition of ripeness to flower, (2) formation of flower primordia, and (3) development of flower clusters and elongation of the axis. He found that light is the dominant factor in determining all three stages. More recently Garner and Allard (1920) have published their opinion that the three primary factors that enter into the action of light upon plants are (1) intensity of the light, (2) quality, that is, the wave length of the

radiation, and (3) duration of exposure. They conclude that the relative length of day is a factor of prime importance in the growth and development of plants, particularly with respect to sexual reproduction, and in 1922 they confirmed and amplified their work. I have been able to confirm this work to a certain extent. A culture of plants growing in the greenhouse was close to an electric lamp used to maintain a constant temperature in a chamber close by, and the plants that were closest to this lamp flowered first, the arc of flowering spreading out centrifugally. After some time all the plants that were near the lamp had flowered, although the rest of the cultures took nearly two months longer to produce flowers. Moreover, the plants that bloomed first were in a comparatively disadvantageous position during the day, so that the effect of the artificial illumination on the flowering of the plants is all the more striking. This observation was repeated in an attempt to hasten the process of flower formation. Two strains of plants, 0215 and 0217, which were both F_1 progeny of crosses made by me, were growing very slowly and were still in the rosette stage by the end of March of this year due to the cold winter. In order to hasten their growth and obtain seed for growing an F_2 population, a few of them were placed three feet below a 300 Watt electric lamp surrounded by a reflector every day from 6 p.m. to 8 a.m. the next day. Some of them shot out flower buds in about three weeks from the time the experiment was started. The rest of the plants in the same families which were not subjected to artificial light had in many cases not started to send up the central floriferous axils. The heat from the lamp may also have had a slight effect.

Moisture.—The plants as they grow in pots in the greenhouse are not subject to much variation in soil moisture because they are watered regularly and the minimum soil moisture necessary for proper growth is usually maintained. The case of the plants grown in the field, however, was different because irrigation water was applied periodically, and owing to the variation in temperature of the days intervening between two successive irrigations, the soil moisture was neither constant, nor was it always above the minimum water requirements of the plants. Consequently, the flowers gradually got smaller as time elapsed after irrigation until, during the hottest part of the day, the plants would show signs of withering. Measurements were taken at this period and showed comparatively the smallest size in the diameter of the capitula. This difference went up usually

to a maximum of 4 mm., but usually it ranged between 2 mm. and 3 mm., and more often reached the lower limit. If at this stage the land was irrigated, the measurements taken the next day invariably showed a rise. The following data taken on plants of the same population both before and after irrigation illustrate this point.

—Culture Hsu 20.1—

Number of plant	Before Irrigation		After Irrigation	
	Number of flowers measured	Average diameter in cm.	Number of flowers measured	Average diameter in cm.
3	4	1.87	5	2.16
9	4	1.97	5	2.22
27	4	1.90	6	2.10
38	3	2.00	5	2.22
49	3	1.96	4	2.25
59	5	1.98	4	2.05
77	4	2.05	5	2.16
99	3	2.00	5	2.16
Total	30	1.95	39	2.16

There is an average difference of 0.21 cm. or approximately 2 mm.

A CROSS INVOLVING DIFFERENCE IN HEAD SIZE

This particular work was started in the summer of 1921 and was carried only to the F_1 stage. Two strains were chosen, one having a diameter ranging from 17 to 25 mm., and the other from 21 to 36 mm. These races had undergone a preliminary purification for size of flower head. The F_1 was intermediate and the mean of the F_1 population was closer to the mean of the smaller parent than that of the larger parent. The data that have been secured on this work are given in table 5. Other crosses have given similar results, but as the parent strains did not differ in any marked degree, the F_1 obtained shows about the same size of head diameter.

TABLE 5
SHOWING RESULTS OF CROSSING FOR DIAMETER OF CAPITULUM

Diam. of heads in mm.	Frequencies		
	H21.1	B21.13	F ₁ hybrids
17	8		
18	74		
19	266		17
20	444		42
21	343	19	85
22	368	53	98
23	278	60	142
24	149	33	103
25	71	36	94
26	24	16	32
27		29	12
28		25	
29		7	
30		10	
31		12	
32		11	
33		13	
34		2	
35			
36		1	
Mean	21.27 ± .027	25.37 ± .131	22.96 ± .048
Stand. Dev.	1.84 ± .019	3.52 ± .093	1.81 ± .036
Coef. Var.	8.6	13.87	7.8

DISCUSSION OF RESULTS

1. The leaves of *Crepis capillaris* vary in outline from a simplex through a scalaris to a bipinnate type of lobing. In the first case, as evidenced by one of the parents used in the cross (fig. 1) the outline is more or less entire, while the other parent in this cross represents the scalaris type. The F₁ progeny obtained exhibited considerable variation but were always intermediate between the two extreme types. In the F₂ there was decided segregation and since only one plant out of over 250 showed characters almost similar to one grandparent, there must be more than one factor responsible for the occurrence of lobes as well as for the number of lobes. The cross 20.1 × 20.13 has

given an intermediate number of lobes in F_1 generation and in F_2 the progeny ranged from one parent type to the other. Out of the 250 F_2 plants studied not one fully represented the grandparent types, and on mathematical considerations there must be at least four factors responsible for this condition. Shull (1918) in his work on the leaf forms of the Shepherd's Purse has formulated a two factor hypothesis, the double dominant homozygote, the two single dominant homozygotes and the double recessive, giving the four classes which he obtained. With regard to the work on the length of the leaf, it has been found that, as compared to the length, the breadth of a leaf is a much more constant character as shown by table 6. The data for this table were

TABLE 6
SHOWING AVERAGE LENGTH AND WIDTH OF LEAVES IN 100 PLANTS OF FAMILY 20,140

Length in cm.	17	18	19	20	21	22	23	24	25	26	27	28
Number of plants	2	6	10	6	6	10	15	13	11	13	6	2
Width in cm.	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6			
Number of plants	13	11	26	27	8	9	0	5	1			

obtained from a family of plants selected at random. This observation is in accordance with the reports of some other investigators. Moreover, the length of a leaf is more markedly susceptible to environmental influences and fluctuations due to modifications will profoundly interfere with estimating the effects of recombinations. It is therefore believed that races should be purified for the breadth factor rather than for the length factors for facilitating studies in this direction.

During the progress of the work, several crosses were made between strains of *Crepis*, and some of the strains were inbred. The result in many cases was comparable to the results of inbreeding in corn. As Collins (1920) has noted, plants of inbred strains may not put out flowers at all, or if they do, very few of the heads set seed. Some of these are viable and give rise to seedlings which may not thrive very well unless they are given special care. They are not as strong as those obtained from hybrid plants. When they have grown beyond the seedling stage, they sometimes stay in the rosette stage much longer than is usual and the vegetative period is consequently prolonged. One strain remained in the rosette stage and produced no flowers although it had been growing for over a year and a half. Other abnormalities have also been noted, such as vegetative proliferation and fasciation of stems and peduncles. Often the flower heads are fasciated and

flattened on two sides assuming the shape of an oval as opposed to the normal round shape and at times, owing to a shortening of the pedicels, two or three flowers appear to be joined together. All these malformations have been noted in one or another of the cultures, and emphasize strongly the effects of inbreeding in bringing to light undesirable recessive characters which are disadvantageous to the growth of the individual plant.

The outcome of this portion of the work has given results in no way contradictory to the conclusions arrived at by other investigators who have relied upon multiple factors as an explanation of inheritance of quantitative characters. As the experiment has not been carried to the F_3 stage, it is not possible to state whether this material will yield results entirely consistent with the requirements (East, 1916) of the multiple factor hypothesis. But as far as the results go, they are in agreement with the explanation suggested that inheritance of the number of lobes in *Crepis capillaris* is a Mendelizing quantitative character and that it is controlled by many factors which affect occurrence of lobes, depth of the incisions, number of lobes, and shape of the lobes.

It may be here noted, in passing, that in a work of this nature a certain amount of discretion is necessary in determining the class to which a given individual belongs. Classification of the shape of a leaf and the exact number of its lobes are, to a certain extent, decided by the investigator, who can handle them quickly as he gains practice. Moreover, the exact times when the measurements are to be taken are more or less fixed by the investigator himself, who should try to secure as uniform material as possible in the several generations. East (1921) has raised a similar point in his work as regards the personality of the investigator. He says, "I believe that in such work as this, the investigator who lives with his plants in the field, who uses all the quantitative data at his command, but who, nevertheless, brings to his aid all the somewhat intangible facts that intimate experience gives him is able to come to a better realization of the truth than one who works on cold data obtained by others."

2. Size of capitulum is a character which is controlled by genetic factors, and it is fairly constant for a given family. It is practically independent of the size of the plant and it cannot fall below a certain minimum. It is also independent of the number of capitula on the entire plant or the number of florets per flower head. It is similarly uninfluenced by the shape of the plant. The tall, erect, vertical type of plants, and the bushy spreading type of plants (pl. 43) have given

sizes of flowers which are practically identical (see East, 1921, p. 329) and while casual observation leads me to believe that the number of flowers per plant and the number of florets per head vary directly with the size and shape of the plant, the diameter of the flower head is not subject to influence by any one of these three factors and is relatively stable. (See Stout, 1918.) The only factor that has been found to influence the size of the flower heads is the moisture content of the soil. The drier the soil the smaller the heads become. Here the plants in pots have an advantage because the soil is never allowed to become dry and the slight variations of moisture to which the plants in pots are subject do not affect the diameter of the flower heads to any appreciable extent. The results obtained from field plants are strictly comparable among themselves, however, since all the strains are subject to the same unfavorable environmental influences and as such give results strictly comparable.

SUMMARY AND CONCLUSIONS

1. *Crepis capillaris* has been found to be a valuable species for genetic investigations because it is a wild plant which has not been subjected to conscious selection by human agency.

2. It can be cross-fertilized and the progeny derived from such cross-fertilization is fertile *inter se* and gives viable seed.

3. Several characters in the plant are constant and breed true when the material has been purified to bring it into a homozygous condition for the character in question.

4. Continual selfing of the plant is followed by the usual symptoms of such treatment in naturally cross-fertilized species, resulting in reduced vitality, arrested development at the rosette stage, formation of many sterile flowers, few viable achenes, vegetative proliferation and fasciation of the capitula and the stem.

5. Three quantitative characters were studied in this plant: the length of the leaf, the number of lobes in the leaves, and the diameter of the flower heads.

6. Length of leaf is a heritable character, but the environment has a very great influence. The resulting fluctuating variability is so great that although crosses have been made for studying the type of inheritance, it is difficult to classify and segregate the F_2 progeny.

7. In inheritance studies, width of leaf is a better index of leaf size than length.

8. Number of lobes per leaf is constant for any given race of plants and the character is determined by four sets of factors:

- (a) The group of factors for presence of lobes.
- (b) The group of factors for depth of the incisions.
- (c) The group of factors for number of lobes.
- (d) The group of factors for extension by which the secondary lobules are developed into lobes.

9. Of these the group of factors for number of lobes consists of at least four interacting factors. The F_1 in these crosses was found to be intermediate and F_2 showed segregation.

10. Races of *Crepis capillaris* with different diameters of capitula were isolated and when crosses were made between such races the diameter of the capitula of F_1 was found to be intermediate between the two parents. The work has not progressed far enough to study the F_2 plants and determine the type of segregation.

11. As far as studied, environment, except moisture, has very little influence on the size of capitula.

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PLATE 42

Fig. 1. Very young stage; cotyledons still persist.

Fig. 2. Early rosette stage.

Fig. 3. Later rosette stage.

Fig. 4. Nearly mature rosette in a family showing a characteristic retrorse rolling of the leaf margins.

Fig. 5. Fully developed rosette, the stage in which measurements of length of radical leaves were taken.



Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5

PLATE 43

Fig. 1. Fully developed plant of spreading habit, i.e., having many divaricate branches arising from the base of the axis. Fully open capitula shown.

Fig. 2. Nearly mature plant similar to that shown in fig. 1, but of erect habit.

Fig. 3. Mature plant of distinct habit, having no secondary branches arising from the base of the axis.

Fig. 4. Mature plant of spreading habit, but a dwarf in stature. ⁷

Fig. 5. Fully open capitula such as were used in taking measurements of diameter.

Fig. 1

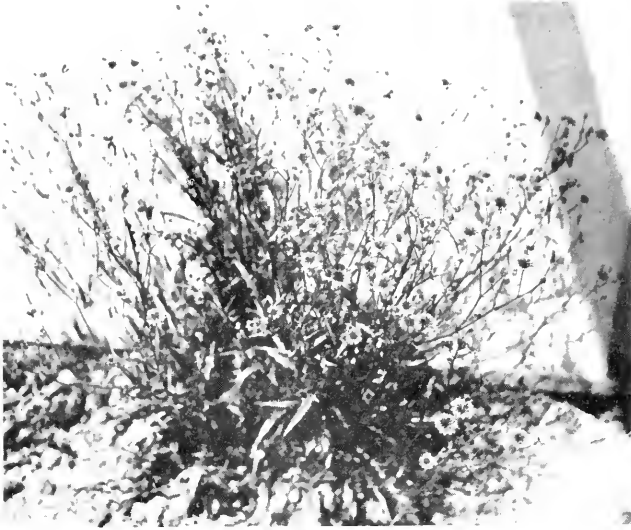


Fig. 3



Fig. 2



Fig. 5

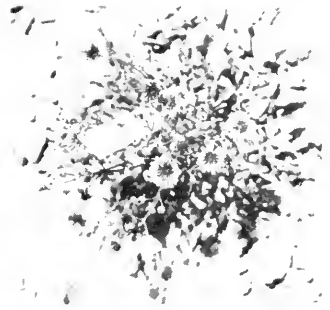


Fig. 4

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September 17, 1924

MICROSPOROGENESIS OF GINKGO BILOBA L.
WITH ESPECIAL REFERENCE TO THE
DISTRIBUTION OF THE PLASTIDS AND
TO CELL WALL FORMATION

BY
MARGARET CAMPBELL MANN

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MICROSPOROGENESIS OF GINKGO BILOBA L.
WITH ESPECIAL REFERENCE TO
THE DISTRIBUTION OF THE
PLASTIDS AND TO CELL
WALL FORMATION

BY
MARGARET CAMPBELL MANN

(Contribution from the Division of Genetics, University of California)

Microsporogenesis in *Ginkgo biloba* L. is especially interesting because the plastids are definitely oriented with respect to the division figures and because they are distributed so that each pollen cell receives approximately one-fourth of them. The cells are large, and both plastids and chromosomes can be observed in living cells. The 12 pairs of chromosomes are nicely separated at late prophase, and one of them is twice as large as the others. This point was previously noted by Cardiff (1906) and Ishikawa (1910).

Smears stained in aceto-carmin were used for most of this study, but smears were also fixed in Flemming's weak and chrom-acetic-urea and stained in iron-haematoxylin, Flemming's triple, and safranin and light green. The plastids are easily observed in aceto-carmin since the large starch grains resist the carmin, remaining a transparent green while the rest of the cytoplasm stains pink, and the chromosomes bright red. The starch grains stain a deep blue in iodine, but show no color in Flemming's triple or haematoxylin. With the former a layer of gentian, with the latter a layer of bluish-black cytoplasm, surrounds each starch grain. Unless one had examined the pollen mother cells before fixation, he might easily interpret the plastid-bearing area in fixed material as an unusually coarse cytoplasmic mesh. This is probably the reason why the phenomena described below have not been previously observed.

OCT 14 1924

The two nuclear divisions precede cell division as in many of the higher plants. It will be seen from the account which follows that the reduction division is typical in all respects.

Before the first, or so-called heterotypic division occurs, the plastids lie between the wall and the nucleus (figs. 1 and 2). As the prophase progresses they enlarge, and some of them divide. The division is usually a simple bipartition, but some appearances suggestive of budding were seen. The cell wall is very thin at first but gradually thickens during the first division. The plastids remain between the nucleus and the wall until after the first spindle has disappeared, when they gradually move, or more probably are moved, between the two groups of late anaphase chromosomes, there forming a ring which nearly fills the space between the two nuclei (fig. 4). This position is retained until after the second division. In the late second ana- or early telophase a portion of the ring of plastids is drawn between each of the two sets of daughter nuclei (fig. 7). The ring then breaks in four places, so that one quarter of it, and consequently about a fourth of the plastids, come to surround the inner face of each nucleus (figs. 7 and 8). The outer wall now pushes inward between each of the four nuclei and finally separates the pollen cells (fig. 9). Before the inpushing of the outer wall two cell plates form at right angles to each other. The method of cell division in *Ginkgo*, then, is a combination of cell plate formation and cytokinesis. The inner portion of the wall (which lies next to the plastid-bearing area) thickens greatly while the outer wall remains unchanged. As the inner wall attains its final thickness, the plastids become generally distributed between it and the nucleus, and a new wall, staining in gentian-violet, appears about each pollen cell. The old wall stains in orange or in light green. As the pollen cells grow they burst the thin outer walls of this case, leaving an empty shell. The plastids now appear smaller, since the amount of starch in them is considerably reduced.

Juranyi (1872, pl. 31, fig. 13) figured a light band between the two daughter nuclei of the pollen mother cells of *Ceratozamia*, which is very like the position of the starch grains of *Ginkgo* at this stage. Sprecher (1907, p. 155) figured the cell plate formation of the pollen mother cells of *Ginkgo*, but neither figures nor mentions plastid or starch grain distribution. Moore (1903) figured plastids like those of *Ginkgo* in the pollen mother cells of *Pallavicinia* but does not discuss their distribution. Smith (1907) shows starch grains in the pollen

tube of *Cycas* which greatly resemble those seen in the pollen mother cells of *Ginkgo*.

The procedure described above raises a number of interesting questions. Firstly, the position of the starch grains bears a definite relation to the formation of the cell walls. They are generally distributed while the first wall is forming, and it ceases to thicken when they withdraw. They lie near the forming cell plate, and near the thick inner wall during its formation. Finally, they become generally distributed during the formation of the pollen cell wall. They are also smaller than they were during the formation of the thick inner wall of the pollen case. It seems possible that they may provide the reserve material which is utilized in wall formation.

Secondly, the method of cell wall formation differs from that common to the higher plants. This is of particular interest on account of the phylogenetic position of *Ginkgo*.

The changes of position of the starch grains are essentially the same as those noted by Terni (1914) for the chondriosomes in the spermatogenesis of *Gecotriton fuscus*, and by Payne (1916) for certain scorpions. The mitochondrial mass forms the tail sheath in such spermatozoa. It would be interesting to know the fate of the plastids in spermatozoa formation of *Ginkgo*.

The similarity of behavior of the chondriosomes during cell division to that observed for the starch-filled plastids of *Ginkgo* indicates that the distributing mechanism is very similar in each case. It does not seem necessary to postulate a separate mechanism for this purpose, the forces already in action being of a type which would, it seems to me, bring about essentially the observed distribution. Whatever the force or forces may be by which the chromosomes are distributed during reduction, the direction of movement of the chromosomes shows the direction in which these forces act. One would expect that a large number of movable cytoplasmic structures or inclusions would be equally distributed between the cell wall and the nucleus, if in- and out-going currents maintained an equilibrium during the early prophase. After the spindle has formed, and the cell is a bipolar structure, the forces (which we may think of as currents) move toward the poles, and presumably back toward the equator. The changes of position of the plastids at this stage indicate such lines of force. As the nuclei grow, apparently by taking in fluid, their increase in size would also tend to force the plastids out of the polar and into the equatorial

regions. The barrel-shaped spindle would hold them in ring formation. When the nuclei have attained their final size, the plastids, now filled with starch, form two rows with the cell plate between them. With the formation of the second spindles this line of division is obliterated. It is possible either that the processes involved in cell plate formation produce the line of division, or that it results from the action of the opposing forces concerned in chromosome division. In any case, the distortion of the ring at late second ana- and early telophase might result from the pull of the same forces which separated the chromosomes. The final division of the plastids into four groups may depend somewhat upon the invaginations which give rise to the lateral walls of the pollen case. In the pollen cell the plastids again revert to the position which they had at early prophase of the pollen mother cell.

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PLATE 44

The drawings are semi-diagrammatic. They were made with a camera lucida, using a 4 mm. dry objective and a number 18 Zeiss compensating ocular. The chromosomes and nuclei are simply outlined, the plastids at the upper focus are in gray wash with a stippled margin, while the lower ones are left white. The drawings show the position of the plastids at successive stages in microsporogenesis.

1. Polar view of late first prophase showing 12 pairs of chromosomes, one of which is about twice the size of the others.

2. Lateral view of same stage showing that the spindle area is largely free of plastids. Most of the plastids are near the poles.

3. Lateral view of late anaphase showing movement of plastids from poles toward equator of cell.

4. Late telophase. The plastids are now arranged in a double ring at the equator.

5. Late second prophase. The plastids are so closely massed that ring formation cannot be distinguished.

6. Late second anaphase. The ring of plastids is being distorted.

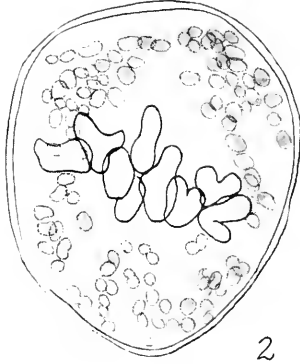
7. Second telophase. The ring is now breaking into four segments, one group lying about the inner face of each of the four nuclei.

8. Early cell division. The dotted lines show cell plate. The outer wall is pushing inward between each of the four nuclei.

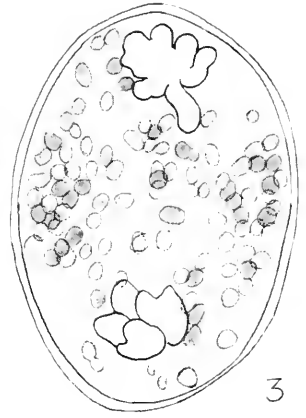
9. Cell division completed.



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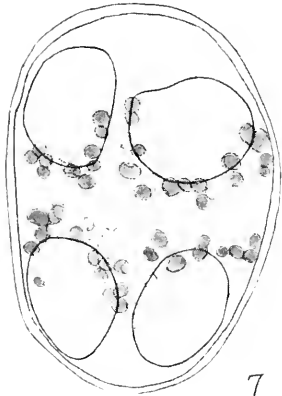
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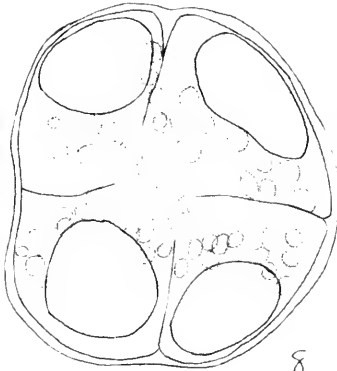
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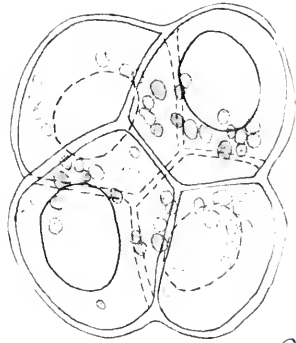
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December 31, 1924

INHERITANCE IN *CREPIS CAPILLARIS*
(L.) WALLR. III.

NINETEEN MORPHOLOGICAL AND
THREE PHYSIOLOGICAL CHARACTERS

BY

J. L. COLLINS

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INHERITANCE IN
CREPIS CAPILLARIS (L.) WALLR. III.²NINETEEN MORPHOLOGICAL AND THREE
PHYSIOLOGICAL CHARACTERS¹

BY

J. L. COLLINS

INTRODUCTION

For several years variations in *Crepis capillaris* have been studied genetically. The study was commenced² in the hope of being able to determine whether the extensions of the Mendelian theory of heredity which were based on breeding data from *Drosophila melanogaster* would hold for higher plants. For this purpose it was necessary to know the mode of inheritance of a number of characters. This paper is concerned with the description and mode of inheritance of a number of variations found in *Crepis capillaris* (L.) Wallr.

It is evident that the material chosen for such a purpose should show variation of a hereditary nature and should also contain a low number of chromosomes. *Crepis capillaris* seemed to fulfil these requirements, for its chromosome number, 3 pairs, is the lowest reported for the higher plants, and the species is known as a variable one.

Linkage has been demonstrated in a number of plants and in some of the higher animals. Unfortunately, the chromosome number in those species in which linkage has been observed is relatively high, and in no case is the number of groups of linked genes equal to the haploid number of the chromosomes.

¹ This is a report on a part of a project supported by appropriations from the Adams Fund.

² Studies commenced by Professor E. B. Babcock in 1915 and carried on by the writer under his direction since 1918; published as nos. 6 and 7 of vol. 2 in the present series.

MATERIAL AND METHODS

The genus *Crepis*, containing over 150 species, is a member of the Cichorieae or chicory tribe of the Compositae, the best known related genera being *Hieracium*, *Lactuca*, *Sonchus*, and *Taraxacum*.

Crepis capillaris (L.) Wallr. is an annual, but under certain circumstances may assume the biennial habit. The plant first produces a rosette of radical leaves which have been found to vary in different plants from entire to bipinnately compound. The stem is usually single with paniculate branching above and varies from a few inches to four feet in height, largely depending upon conditions of growth. The cauline leaves are sessile, amplexicaul, clasping, the lower ones more or less lobed or pinnatifid, while the upper ones are slender and entire. The underside of the midribs of the rosette leaves, and to some extent the upper side, and the lower cauline leaves are more or less covered with bristly hairs. In many, but not all, plants the involucre and peduncle are glandular pubescent in addition to the fine gray tomentum which is always present. The brown terete achenes vary in length from 2 to 3 mm., are attenuate at both apex and base, and usually 10-ribbed. The yellow flower heads vary from 17 to 25 mm. in diameter.

During the course of the investigations, achenes of *C. capillaris* have been obtained from many localities of the temperate and subtropical zones of both the old and the new world. The species is apparently a native of Europe, but is now disseminated throughout the world.

The methods used in growing experimental cultures of *Crepis* have been previously published (Collins, 1922).

In presenting data from hybrid populations, the degree of correspondence of observed with calculated distribution has been determined by use of tables of probable errors of Mendelian ratios prepared by the Department of Plant Breeding of Cornell University. In the case of some dihybrid populations the method suggested by Harris (1912) has been used. This formula is $X^2 = \sum \frac{(o - c)^2}{c}$, in which o is the observed frequency of any class, c , the calculated frequency for that class, and \sum indicates that all the values of the type $\frac{(o - c)^2}{c}$ are added together. From Elderton's³ tables for calculating the goodness of fit, the probability for the chance occurrence of the deviations in the observed classes has been obtained from the calculated value of X^2 .

³ Given in Pearson, K., *Tables for Statisticians and Biometricians*, Cambridge Univ. Press, 1914.

VARIATIONS IN CREPIS CAPILLARIS

Observations upon cultures grown from the achenes obtained from localities in many different regions have resulted in the discovery of a number of variations. Those which have been studied sufficiently to show their method of inheritance are described below. In assigning symbols to serve as genetic representatives of particular characters, the system in general use has been followed, namely, the use of the initial letter (or letters) of the name given to the character, small letters indicating a recessive, and capital letters a dominant condition.

BALD (b)

On August 17, 1918, a single plant (19.18P₂₃) in a culture of 47 plants grown from achenes sent from Copenhagen was found to be devoid of glandular pubescence on the involucre and peduncle. This variation has been named 'bald.' The second instance of this variation was in the same race but appeared only after two generations of inbreeding. Bald plants later appeared in cultures from other localities as follows: Sweden, England, France, Chile, and the Azores. It was of importance to know whether the same or different genes were responsible for the appearance of 'bald' in cultures from such widely separated sources. This could be determined by crossing the different races. If a single gene were involved, then bald F₁ plants should result, while if, on the other hand, glandular plants resulted in the F₁, this variation appearing in the different stocks would be the similar expression of different genes. As is shown in table 1, the same gene is present in each case.

TABLE 1

THE F₁ RESULTS OF CROSSING DIFFERENT GEOGRAPHICAL RACES OF BALD

Culture No.	Character of F ₁		Total
	Bald	Glandular	
F ₂ Copenhagen × Sweden (20.130) × Chile (21.23)...	9	0	9
Sweden (19.235) × Cambridge (19.66).....	4	0	4
Copenhagen (18.75) × Sweden (19.235) (19.H1, 20.57-8, 21.101).....	56	0	56
Chile (20.36) × Azores (20.40), (21.25)	1	0	1
Sweden (19.H3) × Azores (20.40), (21.117).....	7	5	12

In the last item in table 1 both bald and glandular plants are recorded. This is as it should be, for the 19.113 plant was an F₁ glandular plant produced by crossing the Swedish bald race (19.235) with a Eureka glandular race (19.224). If the bald gene in the

TABLE 2
F₁ RESULTS FROM CROSSES OF BB × bb

Pedigree No.	Character of F ₁ plants	
	Glandular (B)	Bald (b)
19.H3	2	0
20.59	10	1
21.21	7	2
21.28	7	0
Total	26	3
Expected 1:0	29	0

TABLE 3
BACK CROSSES OF THE F₁ Bb to bb

Pedigree No.	Progeny segregation	
	B	b
21.17	7	3
21.18	5	6
21.19	6	7
21.24	4	2
21.117	12	13
21.126	4	2
Total	39	38
Calculated 1:1	38.5	38.5
Deviation	0.5 ± 3.84	

cultures from Sweden and from the Azores were identical, we should expect to obtain from such a back cross 50 per cent glandular and 50 per cent bald plants. The 5 to 7 segregation obtained is a close approximation to the expected 1 to 1 ratio. While the Copenhagen race has not been crossed with the Cambridge race, nor the Chilean

race with any except that from the Azores, we have evidence of their identity, since they have each been crossed with the Swedish race, which in turn was proved to be identical with the others. The bald plants from France have not been tested. Bald is inherited as a simple monohybrid recessive, as is shown by the results obtained from crossing with glandular plants. Table 2 presents F_1 data from crosses of bald \times glandular. The one bald plant in culture 20.59 probably resulted from the failure to remove a single pollen grain during emasculation and represents an error in technique. The two bald

TABLE 4
F₂ RESULTS FROM THE CROSS BB \times bb

Pedigree No.	Progeny segregation	
	B	b
20.59	10	1
20.60	2	3
20.141	56	17
20.142	16	7
20.118	74	23
Total	158	51
Calculated 3:1	156.75	52.25
Deviation	1.25 \pm 4.22	

plants in culture 21.21 may be ascribed to this same cause or to errors at time of transplanting, since culture 21.23, containing only bald plants, grew adjacent to 21.21 in the flat before transplanting to the field.

Table 3 shows that 39 glandular to 38 bald plants were obtained when the F_1 (bald \times glandular) were backcrossed to the recessive parent strain. The expected 1 to 1 ratio was therefore realized.

The results from F_2 cultures confirm the conclusion regarding a single recessive factor conditioning the appearance of bald. While in almost all cases involving bald the glandular hairs are completely absent, in culture 20.141 some plants appeared to be somewhat intermediate, inasmuch as they developed a few small scattered gland hairs on the involucre. They were easily distinguishable from glandular plants. In table 4 these intermediates have been classified as bald,

but in the original records they were designated as intermediates. If the culture 20.141, containing the intermediate-bald plants, is removed from the table, the remaining cultures give an exact ratio of 3 glandular to 1 bald; when the intermediates are classified as bald, the deviation from a 3 to 1 ratio is less than the probable error. The progeny of two bald and two glandular F_2 plants were grown. Both of the former gave, as expected, only bald offspring, while the two glandular F_2 plants produced both types in F_3 .

The nature of the intermediate plants has not been definitely determined. The selfed progeny from one plant (18.d1P₇₆) gave a culture (20.55) of 18 bald, 3 intermediate, and 3 glandular plants. That they were not due to the incomplete dominance of the hybrid produced by crossing bald with glandular is certain, for in the F_1 cultures (table 2) all plants were fully glandular. Another intermediate bald plant (22.153P₁₈) produced 5 glandular and 5 bald plants from selfed seed but none that could be classified as intermediate.

SMOOTH MIDRIBS (s)

The midribs of the rosette leaves usually have a hairy pubescence. From sporadically appearing plants, races have been obtained which do not show these rib hairs; such plants have been designated as 'smooth' (s). The F_1 resulting from a cross between these two types of plants were all rib-haired, and in the F_2 there appeared 556 rib-haired to 40 smooth plants. This is approximately a 15 to 1 ratio and suggests the operation of two independent genes, each producing the same somatic effect.

Duplicate genes are by no means unknown, having been reported a number of times in the literature of genetics. If two independent genes were operating in the cultures 21.140 and 21.141, the F_1 of this same cross when backcrossed to smooth should give a 3S to 1s ratio and some F_3 populations should give a 3 to 1 segregation. Evidence from cultures of these two types has been obtained; the data from them together with data from other crosses involving this character are given in table 5. The F_3 culture 21.189 was grown from one plant of an F_2 culture containing 58 rib-haired and no smooth plants. Such a deviation is, however, only three times the probable error and may well be due to errors of random sampling. The culture F_1 19.III was originally made to determine the relation of the gene for bald of the English race of *Crepis* to that in the Danish race and

was the hybrid between these two races. The parent plant from the English race was smooth, while the parent from the Danish race had rib hairs.

TABLE 5

SHOWING F₂ AND F₃ RESULTS FROM THE CROSS SSS'S', SSS's', AND SsS's'
WITH sss's'

Pedigree No.	Progeny segregation	
	S	s
F ₂ 21.140	237	17
F ₂ 21.141	319	23
F ₃ 22.189	189	9
Total	743	49
Calculated 15:1	742.5	49.5
Deviation	0.5 ± 4.59	
F ₂ 22.55	25	12
F ₂ 22.56	5	2
F ₂ 22.60	22	6
F ₂ 22.61	4	2
F ₂ 22.62	9	1
F ₂ 22.63	34	8
F ₂ 22.41	66	24
Total	165	55
Calculated 3:1	165	55
Deviation	0.0 ± 4.52	
Back cross 19.H1	55	17
Calculated 3:1	54	18
Deviation	1.0 ± 3.83	

The 3 to 1 ratio obtained in 19.H1 indicates that the rib-haired ♀ used was heterozygous for the duplicate genes for rib hairs. This cross, as regards these characters, was a back cross of a heterozygote to the recessive parent, and constitutes additional evidence to substantiate the duplicate gene interpretation given above for the inheritance of rib hairs in these cultures.

LEAF VARIATIONS

From the very first acquaintance with *C. capillaris*, the different forms in the rosette leaves constituted the most striking and outstanding variations. They have proved equally as difficult to study genetically, due, first, to the difficulty in evaluating non-genetic variability resulting from age of plant and from environmental causes, and, second, to the complex heterozygotic nature of the material in the wild condition. Sears (1921) found in *Taraxacum* that the degree of leaf dissection is correlated with the age of a given rosette. The leaves of a very young rosette are almost entire, becoming progressively more dissected as the rosette becomes older. Stork (1920), also working with *Taraxacum*, found that in very young plants the rosette leaves ranged in form from entire to deeply pinnatifid-runcinate, but became *more, instead of less uniform*, as they grew older. Neither condition can therefore be taken as typical for that species. In *Crepis*, a closely related genus, there is a more regular sequence of development of leaf shape for a particular rosette. The juvenile leaves are usually entire or nearly so, and assume their typical forms gradually as the plant reaches the mature rosette stage just preceding the appearance of the flowering stalk. At this time there exist individual differences which range in form from entire to deeply pinnatifid or compound pinnatifid. That these differences are genetic is shown, first, by the fact that inbreeding has resulted in the isolation of races of the different types which breed true when grown side by side under similar conditions, thus to a large degree eliminating the effect of the non-genetic factors, and, second, that the forms when crossed give a fairly uniform F_1 and segregate into the parental and F_1 forms in the second generation.

By means of inbreeding and selection, a number of distinctive, uniform races have been obtained in almost homozygous condition. A brief description of each is given below.

VIRIDIS

Plate 45, figure 1

This form was isolated in 1919 from the Eureka (California) stock. The rosettes are small, 4 to 10 inches in diameter. The leaves are deeply lobed or pinnately parted, and are lacking in anthocyanin.

The blade of the leaf is of a darker color than the midrib. The color of the blade is Ridgway's varleys green, 31' m. The midrib is covered on both upper and lower surfaces with hairy pubescence. The lobes are usually widest at the base, often having a minor lobe attached to the proximal edge of the base of the major lobe. Attached to the midrib between the lobes is a narrow wing. The lobes are usually close together, with the terminal lobe slender and pointed.

H6 RACE

Plate 45, figure 2

The H6 race was isolated in 1919 from a Berkeley *Crepis* stock. The size of the rosettes is more variable than in viridis, the rosettes ranging from 8 to 12 inches in diameter. The leaves are pinnately and bipinnately lobed; the lobes are constricted at the base and rounded at the tip, and inclined to twist, so that the plane of the lobe is not in the same plane with the midrib. Anthocyanin is conspicuously present. There are no hairs on the midrib. The lobes, usually six in number, are widely spaced. The terminal lobe is large and blunt-tipped. The narrow wing on the midrib is crimped, presenting a ruffled effect. The wing and edges of the lobes contain a blackish purple coloring which appears very early in the development of the plant. The leaf color, according to Ridgway's Standard, is cedar green, 31m. The characters which make up this type are dominant, excepting smooth ribs, when crossed with viridis.

PALLID

Plate 45, figure 1

This race was obtained in 1919 by inbreeding in the same Eureka stock that produced the viridis race. The rosettes are from 6 to 10 inches in diameter. This race produces more leaves in the rosette than do the preceding races, giving the rosette a thick mat-like appearance. Pallid lacks anthocyanin and is a much paler green (Ridgway's forest green, 29'm.) than the two races described above. The lobes are broadest at the base, are set closely together, and have pronounced, pointed teeth. This race does not grow so rapidly as the darker green races. Rib hairs are present on the midrib.

SIMPLEX Z9

Plate 46, figure 1

Simplex Z9 was isolated in 1920 from a stock originating from seed collected at Quy Fen, England. The original culture consisted of plants ranging from entire to pinnatifid. The simplex Z9 race was obtained by inbreeding plants with entire leaves. Although inbreeding has reduced the amount of variation, there still appears in this supposedly homozygous race a small percentage of semi-pinnatifid-leaved plants (pl. 46, fig. 1). Anthocyanin and rib hairs are present.

SCALARIS e29

Plate 46, figure 2

This race was isolated in 1919 from the Eureka stock of *Crepis* which produced the viridis and the pallid races. It is characterized chiefly by long, simple, pinnately-divided leaves with pointed lobes. The terminal lobe is slender and elongated, often curved to one side near the tip. Both anthocyanin and rib hairs are present. The average number of lobes per leaf is 10. It is dominant when crossed with simplex Z9 or with viridis. Typical leaves of the scalaris e29 and the simplex Z9 races are shown in plate 52, together with the F_1 and F_2 types obtained when these two races are crossed. In the F_1 a few extreme variants occur which approach the simplex form, but the majority are more nearly like the scalaris and constitute a fairly uniform intermediate type. In the F_2 , three types are distinguishable (see pl. 51, fig. 2), the two grandparental forms and an intermediate scalaris form similar to the F_1 . When the intermediate-scalaris and the scalaris are grouped together a 3 to 1 ratio is obtained (see table 6). The intermediate forms differ from the scalaris in having the lobes less deeply incised, some more so than others, but still classifiable as intermediate. (See third and fourth leaves in F_2 , pl. 52.)

From the results of breeding it appears that there is present one main gene for lobing and that dominant modifying genes are involved which act cumulatively, thus producing intermediates of different grades of pinnate lobing. As a corollary to this hypothesis races breeding true for different grades of intermediate pinnatifid lobing should be possible. There is evidence that such races occur. Several intermediate forms have been tested and found to be fairly constant.

A race obtained from Seattle, Washington (named "Seattle") appears to be such a homozygous intermediate form.

Races of *Crepis capillaris* also differ in number of lobes per leaf and in length of leaf (Rau, 1923). The scalaris race shown in plate 52 has a large number of lobes. The two races differ, however, in length of leaf. The leaves of the scalaris parent shown in plate 52 are shorter, and of the simplex parent larger, than the mean size typical for each race. The F_1 is usually larger than either parent. The F_2 in the same figure shows the segregation for size which appears to be due to multiple genes.

The inheritance of pinnatifid and entire leaf forms in *capillaris* conforms in general to the type of inheritance of corresponding forms in a number of other plants. Rasmusen (1916) found in species crosses in grapes that differences in leaf form behaved in a very similar way. The F_1 appeared to be intermediate between the shapes of the parent leaves. In the F_2 , a series was produced which included the grandparental forms, the F_1 type and different grades of intermediates. If the deeply toothed and intermediate toothed classes were grouped together, a ratio of 3 toothed to 1 non-toothed resulted.

Shull (1918) found four different leaf forms of the shepherd's purse to be caused by two pairs of factors. As in *Crepis*, the deeply pinnatifid forms were dominant. The plants were also subject to considerable fluctuating variation. Two races of *Urtica*, one having deeply serrated leaves, the other, leaves with entire edges, gave serrated leaves in F_1 and a ratio of 3 serrated to 1 entire leaf in the F_2 generation (Correns, 1912). In cotton, however, the deeply palmately parted leaf form is not dominant when crossed with the five-pointed upland type, but produces an intermediate type in F_1 with a ratio of 1:2:1 in the F_2 generation (Shoemaker, 1909). Kristofferson (1923) found that the difference in lobing of the leaves of two species of *Malva* was brought about through a single genetic factor, and resulted in a somewhat intermediate condition in F_1 and a 3 lobed to 1 non-lobed condition in the F_2 , although considerable variation in the degree of lobing in the pinnatifid class was recognized. Tedin (1923), on the other hand, found that pinnatifid and entire leaved plants differed genetically by two factors.

TABLE 6
THE RESULTS FROM THE CROSS OF LEAF FORMS. Sc × sc

Pedigree No.	Progeny segregation	
	Sc	sc
21.140	177	75
22.7	99	24
22.10	50	17
22.14	14	6
22.17	48	15
22.19	92	19
22.22	167	52
22.24	51	14
22.25	37	13
22.26	29	2
Total	764	237
Calculated 3:1	750.75	250.25
Deviation	13.25 ± 9.24	

SCALARIS e28 (Sc)

Plate 47, figure 1

This pinnatifid leaf form was isolated in 1919; it originated from a single plant which was a sib to the one producing the scalaris e29 race. These two forms have much in common, but are different in size, e28 being smaller and not so vigorous as e29, and having shorter and blunter lobes.

Two races of the pinnatifid leaf forms isolated from the Berkeley (H6) race of plants and from the Eureka population (e28), respectively, differ in a number of minor characters, as shown in the following comparative list:

H6 (BERKELEY)	CHARACTERS	e28 (EUREKA)
dark green	color of leaf	dark green
dark green to blackish	color of midrib	light green
pronounced	anthocyanin	none or trace
pronounced	crimping of rib-wing	none
none	rib hairs	present
pronounced	black edge on leaf	trace only
blunt and rounded	terminal lobe	narrow—pointed
rounded	lateral lobe	slender—more pointed
wide (very)	lobe spacing	wide (medium)
pronounced	Constricted base of lobes	none or trace
large	secondary lobes	none

Plants of these two races when crossed showed almost the entire group of H6 characters (rib hairs excepted) in the F_1 , while in F_2 (21.141) there appeared the parental types and in addition some composite types that showed some characters from each parent. When each character pair was considered separately, however, a peculiar situation was presented. Six of the character pairs gave 9 to 7 ratios, and a seventh pair, rib hairs vs. smooth ribs, gave a 15 to 1 ratio. The data for these characters are included in table 7. It is quite probable that these six character pairs as given are the result of not more than three sets of genes, since the two characters, black edging of the leaves and anthocyanin of the midribs, are both concerned with the distribution of anthocyanin pigment in the plant. The shape of the terminal and of the lateral lobes is probably conditioned by the same pairs of genes, while the crimping of the wing of the midrib and the constriction of the base of the lobes also probably result from the action of the same gene. The Berkeley plants were evidently homozygous for the dominant complementary genes of all three character couples. This genotype may be expressed as AA'BB'CC', the simultaneous presence of both the primed and unprimed dominant genes being necessary to cause the development of the respective characters. The Eureka race would then have the genotype aa'bb'ee' with respect to these characters.

TABLE 7

SEGREGATION OF SIX PAIRS OF CHARACTERS IN THE F_2 FROM THE CROSS
H6 X SCALARIS e28. (CULTURE 21.141)

Segregation	Calculated 9 : 7	Deviation
162 black edge : 103 green edge.....	149.06 : 115.93	12.94 ± 5.45
166 anthocyanin : 109 none.....	154.71 : 120.33	11.29 ± 5.55
142 angular lobes : 112 round.....	143.1 : 111.3	1.1 ± 5.33
150 narrow lobes : 104 broad lobes.....	143.1 : 111.3	6.9 ± 5.33
135 constricted lobes : 118 non-constricted.....	143.1 : 111.3	8.1 ± 5.32
165 crimped wing : 101 flat wing	149.58 : 116.34	15.42 ± 5.49

REVOLUTE (r)

Plate 47, figure 2

This race appeared in 1919 among offspring of a plant of the Eureka stock, which had been self-pollinated. The plants are characterized by a definite downward curling of the edge of the leaf

toward the midrib. It occurs in both entire and pinnatifid types, though it is more conspicuous in the former. In appearance much like the *funifolia* mutant of *Oenothera Lamarckiana* described by Shull (1921), in which both rosette and cauline leaves have edges curled under. The knowledge of the genetic basis for this character has been obtained incidentally in experiments designed to show inheritance of other characters. The data thus obtained indicate that revoluteness is conditioned by complementary recessive genes.

TABLE 8
SHOWING THE SEGREGATION OF REVOLUTE LEAVES IN TWO CULTURES

Pedigree No.	Progeny segregation	
	R	r
19.c5 Calculated 3:1	62 59.25	17 19.75
Deviation	2.75 ± 2.60	
21.140 Calculated 15:1	233 237.19	20 15.81
Deviation	4.19 ± 2.60	

It is significant that revolute appeared only in these two cultures, which were derived from a common source, because it indicates that the genes were present in the wild plants from which the starting point of these cultures was obtained. The 15 to 1 ratio made its appearance in the sixth generation from the wild plants (some out-crossing occurs in this pedigree), while the 3 to 1 ratio appeared in the second generation.

BICEPHALIC (bi)

Plate 48, figure 1

This character designates a type of fasciation in which the buds are more or less joined together in twos. The peduncle is also frequently flattened. This variation was first found in 1920 on a single plant (20.30) which was grown from achenes obtained from Chile. This original plant was crossed with 20.130P₁₅, which produced an F₁ culture of 9 normal plants. The F₂, consisting of 81 plants, segregated into 60 normal to 21 bicephalic, clearly a monofactorial ratio.

In no case were all the buds of a plant of the bicephalic kind. Some plants indeed produced only a few double buds. F_2 bicephalic plants of both types were selfed and F_3 cultures produced. The data from F_3 cultures are shown in table 9.

TABLE 9
TYPE OF PLANTS PRODUCED BY SELFING F_2 BICEPHALIC PLANTS

F ₂ Plant No. 23.283	Progeny F ₃	
	Bicephalic	Normal
*P ₆₈ +	6	1
P ₇₀ +	2	6
P ₉₆ +	8	0
P ₂ ++	6	0
P ₁₀ ++	6	0
P ₂₃ ++	5	1
P ₂₄ ++	2	0
P ₃₀ ++	0	1
P ₄₄ ++	20	0
P ₄₆ ++	8	0
P ₄₈ ++	5	2
P ₅₇ ++	2	0
P ₈₁ ++	5	(2?)

* The single + indicates an F_2 plant on which but few bicephalic buds appeared. The ++ indicates plants having many such buds.

It appears that F_2 bicephalic plants breed true in F_3 . Plant 70 which had only a few double buds, was apparently a heterozygote, for it gave a 3 to 1 ratio in F_3 . The other F_3 plants listed as normal may have been genetically bicephalic, since they showed some evidences of fasciation in the stems and malformation of buds; but no doubling or cohesion of the buds was found.

ANTHOCYANIN

This pigment is distributed to many parts of the plant, but is most noticeable in the midribs of the leaves and on the lower portions of the stems. Culture 19.e8 segregated into 94 plants with anthocyanin to 39 with none or developed only to a slight degree. The ratio in this case is 2.82 to 1.17, in which the deviation is less than twice the probable error. This segregation can be considered only as suggestive because of the difficulty of accurately classifying this character

in *Crepis*. The appearance of purple anthocyanin color depends upon a certain amount of sunshine and exposure to light. Plants known to be capable of producing the color will show it to only a small degree if conditions for anthocyanin development are adverse, while, on the other hand, races in which it does not normally appear conspicuously will produce it under conditions of sudden exposure to direct sunshine or sometimes as a result of mutilation caused by animals or insects. The development of anthocyanin is a matter of degree, for the potentiality for its development is not entirely absent from any race so far obtained. In the *viridis* race we have it in its lowest and in the H6 race in its highest development. Crosses between high and low anthocyanin races (other than 19.e8 mentioned above) in general produced F_1 plants showing the darker anthocyanin of the H6 race, but in F_2 produced a series of forms showing a gradation in pigment from one parent to the other. In most cases the parental types were also duplicated. One such cross, H6 \times *viridis* e33, gave an F_1 more nearly like the H6, but in F_2 the types were distributed as follows: 9 of H6, 3 of *viridis*, and 3 distinctly between these two parental types. The segregation of anthocyanin has been observed in other cultures (e26 = 3 to 1), but has not, in general, given sufficiently regular results to warrant the drawing of conclusions regarding its genetic basis. The analysis can only proceed when facilities are available to control more accurately the environmental factors which alter its development.

DWARF II (dII)

Plate 48, figure 2

This variation first appeared in culture 21.99, which was the second selfed generation from achenes obtained from Lyons, France. It is characterized by a very small rosette of slender semi-scalaris leaves which are blotched with yellow and yellowish red coloration, giving them the appearance of being about half-dead. Due to their peculiar appearance the first plants were thought to be suffering from poor environment, although adjacent plants were healthy. The plants when mature are very small (3-6 inches in height), the stems very fine and spreading. In the first culture the dwarf effect appeared to be recessive (5 dwarfs in 16 plants) and bred true in the next generation. Culture 22.159 from 21.99P_{7s}, a normal plant, contained 51 plants, 3 of which were dwarf II and 3 somewhat dwarfish but not typical for dwarf II. This is approximately a 15 to 1 ratio, and

indicates that there may be duplicate genes for dwarf II; sufficient data are not at hand to establish the hypothesis. Culture 22.160 (from 21.99P₁₅, a normal plant) gave 84 normal plants.

The yellow appearance of the leaves in dwarf II seems to be a dominant character from its appearance in 22.407, F₁ of the cross 22.169P₂₂ × 22.261P₄, the male parent being a dwarf II plant from a pure culture. Inasmuch as the F₁ plants are not dwarfish, it appears that the yellowing and dwarfing may be due to separate but probably linked genes. All the dwarf II plants which have appeared were yellowish, and we may therefore assume that, instead of linkage, the appearance of dwarf II is dependent on the presence in the zygote of the dominant gene causing yellowing.

DWARF III (dIII)

Plate 49, figure 1

This variation first appeared in 1919 culture e5. It reappeared in 1921 in a culture (21.76) which came from the same source as e5. The ratio of normal to dwarf III in 21.76 was 15 to 1, and in the progeny of 21.76P₁ (culture 22.117) 3 to 1. (See table 10 for data.) Dwarf III was at first called 'semi-lethal,' because of the high mortality in this class of plants. These plants remain very much smaller than their normal sibs during the rosette stage and reach maturity much later. A large percentage die after they have formed a rosette and before they reach the flowering stage.

This variation appeared in several members of the same stock which produced revolute, viridis, and pallid.

SPREADING (sp)

Plate 49, figure 2

A lax, open-branching habit which appeared in 20.37, the French stock of *Crepis*. The stems and branches are long and slender, appearing to be so weak they cannot support themselves in upright position. Dwarf II appeared in this race and all have this spreading habit. Data from crosses (21.26 and 22.173, table 10) show that it is a recessive character. When the same plant (20.37P₂) was crossed to another erect plant (19.IIIP₁₁), it behaved as a dominant (21.28, 22.41, and 22.43, table 10). Of the F₂ cultures, only 22.173 was grown under desirable conditions; the others were overcrowded in greenhouse and lath house, which interfered with proper development of this character.

PROCUMBENT (p)

This variation is similar in appearance to spreading. It first appeared in culture 20.40, which came from achenes sent from the Azores Islands. Unlike spreading, it seems to be dominant, the F_1 plants, 21.28 (from 20.40 $P_0 \times$ 20.111 P_1), being of the procumbent type. The F_2 cultures were grown under crowded and unfavorable

TABLE 10
SEGREGATION OF PLANT CHARACTERS

Culture No.	Segregation	
	Normal	Variant
21.76 Calculated 15:1	57 57.19	4 dwarf III 3.81
Deviation	0.19 \pm 1.28	
22.159 Calculated 15:1	48 47.81	3 dwarf II 3.19
Deviation	0.19 \pm 1.17	
22.117 Calculated 3:1	12 12	4 dwarf III 4
Deviation	0.0 \pm 1.17	
22.99 Calculated 3:1	11 12	5 dwarf II 4
Deviation	1.0 \pm 1.17	
22.173 Calculated 3:1	70 erect 72 erect	26 spreading 24 spreading
Deviation	2.0 \pm 2.86	
22.41 22.43	18 5	39 spreading 15 spreading
Total Calculated 1:3	23 19.2	54 57.8
Deviation	3.8 \pm 2.56	

conditions which made accurate classification difficult and uncertain. One F_2 gave a 1 to 1 ratio and another the ratio 2 procumbent to 1 normal.

ERECT (e)

Plate 50, figure 1

A strain characterized by erect habit of growth, large stiff lateral branches, and a thick rigid central axis. The branches make an acute angle with the axis, the whole plant having the form of an inverted cone. This form was selected from the F_2 of a cross between the Danish and Swedish stocks.

PALEA (p)

Plate 51, figure 1

The nature of this character has previously been discussed (Collins, 1921). It originally appeared in an F_1 hybrid and was considered a reversion to a possible, pre-composite, ancestral condition. It has appeared in every case in hybrids, never in inbred races, and was probably introduced with the Danish stock, since the same plant (17.198P₂) of that stock is in the pedigree of all the hybrids which have produced palea. Races homozygous for palea have been obtained. Preliminary data show palea to be conditioned by a single recessive gene.

LINKAGE

In a species having only three pairs of chromosomes, it would seem fairly easy to establish groups of linked genes, especially when the species was known to be more or less polymorphic. However, it has not yet been possible to realize this end, due to the unexpected relations of some of the genes in this species. For instance, there are four cases of complementary recessive genes, and three characters dependent upon duplicate dominant genes. The determination of linkage groups under such conditions is complicated because it requires a longer time to obtain races with a known and tested genotype.

The gene for bald involucre appears from data in tables 12 and 13 not to be linked with the gene for smooth ribs nor with the gene for procumbent, since the ratios show independent segregation.

It is of course obvious that linkage must occur between one pair of complementary genes for smooth ribs and one pair of complementary genes for revolute leaves, since there are four pairs of genes and

only three pairs of chromosomes. A cross involving these two characters gave the following results (+ indicates the presence and — the absence of the character named):

TABLE 11
DIHYBRID SEGREGATION OF SMOOTH \times REVOLUTE IN A CULTURE WHICH GAVE A
15:1 RATIO FOR EACH CHARACTER SEPARATELY

Culture 21.140	Smooth ribs Revolute leaves	— —	— +	+ —	+ +	Total
	Observed	202	16	32	2	
Calculated	224	11.79	11.79	3.93	252	
	57:3:3:1:					
	$\frac{(o-c)^2}{c}$	1.98	1.50	34.64	0.12	$X^2 = 38.24$ $P = .0000$

The calculated numbers agree fairly well with those obtained except in the third class where the observed numbers are more than twice as large as the calculated number. This class may have been increased at the expense of the first class by placing in it some plants which genetically belonged in the latter. The observed number in the first class is considerably less than the calculated number for that class. These figures indicate that the genes are arranged in the three pairs of chromosomes as follows: R, s,—(R'S') (r's')—r, S, where primed genes are the complements of the unprimed genes. Were the linkages as follows (R's) and (r'S), the F_2 population should consist of three classes in the proportion of 14:1:1, assuming that little or no crossing over occurs. A high percentage of crossing over in the latter type of linkage would give approximately the results obtained. It appears, therefore, that either the dominants are linked, as stated above, or that there is a high percentage of crossing over between the linked genes. This inference can be tested experimentally, for races have been obtained which gave 3 to 1 ratios for both of the characters.

EFFECTS OF INBREEDING

The flowers of *Crepis* are perfect and, although self-fertilization can take place, the arrangement of the stigmas in respect to the stamens is such as to permit cross-pollination before self-pollination can be naturally effected. The stamens are united into a tube surrounding the style, and the pollen is shed on the inside of this tube.

TABLE 12

F₂ RESULTS FROM THE DIHYBRID CROSS, GLANDULAR AND HAIRY MIDRIB × BALD AND SMOOTH RIBS, SHOWING INDEPENDENT SEGREGATION

Culture 22.41*	Observed segregation	Calculated segregation 9 : 3 : 3 : 1	$\frac{(o-e)^2}{e}$
Glandular and Rib Hairs	36	41.01	0.61
Glandular and Smooth	11	13.68	0.52
Bald and Rib Hairs	20	13.68	2.84
Bald and Smooth	6	4.56	0.42
	73	72.96	X ² = 4.39 P = 0.2264

*Rib hairs vs. smooth in this culture show a 3 : 1 ratio.

TABLE 13

SHOWING INDEPENDENT SEGREGATION IN F₂ OF DIHYBRID CROSS,
GLANDULAR-ERECT × BALD-PROCUMBENT

Culture No. 22.41	Observed segregation	Calculated segregation 9 : 3 : 3 : 1	$\frac{(o-e)^2}{e}$
Glandular— procumbent	17	20.25	0.37
Glandular— erect	10	6.75	1.56
Bald— procumbent	7	6.75	0.01
Bald— erect	2	2.25	0.03
	36	36.00	X ² = 1.97 P = .5773

The style is bifid with the stigmatic surface on the adjacent faces of the lobes. With the beginning of anthesis the style elongates, pushing the upper end out from the stamen tube and sweeping the pollen out with it on its outer surface. The stigmatic lobes then separate and assume a position at right angles to the style. The pollen at this stage is below the receptive surface of the stigma, which is, however, exposed to insects, the means by which cross-pollination is effected. Later the stigma lobes curl into a short spiral which brings the receptive surface of the stigma in contact with its own pollen or that of an adjacent floret of the same head. Under natural conditions *Crepis* is often cross-pollinated by insects, and this preserves a heterozygosity of the germinal material. A similarity of the effects of continued inbreeding in *Crepis* to the effects of inbreeding in maize has been noted (Collins, 1920). It was shown that inbreeding caused a reduction in the size of the plants and increased the length of the vegetative period. Other data are now available which show in another way the general heterozygosity of *Crepis capillaris* as it occurs in a wild state. Thus the seed collected from a few wild plants near Eureka, California, has been the source of the following races: viridis, scalaris-e28, pallid, and revolute (leaf form variations); of three types of partial albinos (chlorophyll development); and of the variations, dwarf III and fasciation (the plant as a whole). From the Berkeley wild plants we have obtained plants with smooth ribs and the leaf form H6; from England, the leaf form simplex-Z9; from France, dwarf II, spreading, chlorina, and tubular flowers. Palea probably came from the Danish material. As mentioned in another section, bald has appeared independently in the cultures from six different geographical regions. The Eureka stock has produced the greater number of new races. This is not taken to mean that it is necessarily more heterozygous but that many more plants from this source have been under observation. We have presented here an instance of a remarkable germinal diversity in locally developed strains of a single species. Although many of the characters appeared only after hybridization between local races or stocks, the evidence does not, except in a few cases, show these characters to be due to complementary factors. The appearance of bald from such widely separated localities as Chile and Sweden and from other less widely separated localities is of particular interest, for it shows that either a certain locus of the germinal material mutates more readily than others or that all these local races have originated from a single stock

in which this gene was present; the former is, however, more probable, for it has been shown in *Drosophila* (Sturtevant, 1921) that certain loci are more mutable than others. Additional evidence that this is the case is found in the fact that a similar variation, bald, has been found to occur in at least four other species, *C. bursifolia*, *C. biennis*, *C. aspera*, and *C. dioscoridis*. A similar germinal diversity among local races of *Drosophila melanogaster* from equally widely separated localities has not been found, and Sturtevant suggests that this may be due to a frequent transportation of individuals from one locality to another. The chances are probably as great for transportation of *Crepis* seeds along with agricultural seeds as for the transportation of *Drosophila* among fruits.

It is possible that some of these variations might have arisen from mutations occurring in the cultures under observation. A study of the wild plants in the fields about Eureka, however, disclosed the fact that some of the forms obtained in the greenhouse by inbreeding were also appearing there among wild plants. In this material it is impossible to say whether any new recessive variation appeared as the result of a recent gene mutation or the segregation of a recessive from a heterozygous parent stock.

VARIATIONS IN CHLOROPHYLL

A number of different variations involving a loss of chlorophyll have appeared. These variations are evident in the seedling stage, but, unlike the usual albinic condition in seedling plants, most of these albino types develop sufficient chlorophyll as the plant grows to enable the plant to live. One type of pure white seedling always dies in the seedling stage. The other types are either pure yellow or yellowish green. The percentage of seedling mortality in these classes is higher than in pure green seedlings.

A complete analysis of the genetic relations of these different types has not yet been possible, but a sufficient study has been made to warrant a preliminary report in this general account of variations in *Crepis capillaris*.

CHLORINA (C)

Chlorina signifies a chlorophyll deficiency in seedling and mature plants. The middle portion of the leaves of chlorina plants is yellowish, but both tip and base contain more or less chlorophyll and thus it is possible for the plant to function. This character first appeared

in culture 21.99. In 1922 a culture of six chlorina plants was obtained. When these chlorina plants were crossed with normal green plants, the two classes of plants—normal and chlorina—appeared in the progeny in equal numbers, thus indicating that the chlorina plants were heterozygous for green. Self-fertilization of the green resulted in only green progeny. The seedling progeny from self-fertilized chlorina plants consisted of three classes: pure yellow, pale green, and normal green, in the ratio 1 to 2 to 1. The yellow seedlings died, the pale green ones developed into chlorina plants, and the green seedlings produced only green plants. The gene for chlorina is therefore dominant and has a lethal action when homozygous.

TABLE 14
SEGREGATION OF SEEDLING PROGENY OF SELF-FERTILIZED CHLORINA PLANTS

Culture No.	Green	Pale green	Yellow
24 171	46	60	26
24 173	13	17	6
24 174	66	?	33
Total	125	77	65
Observed	202		65
Calculated 3:1	200.25		66.75
Deviation	1.25 ± 4.77		

In table 14 the seedlings in culture 24.174 intergraded in such a way that it was impossible to make an accurate segregation of pale green from green; consequently the two classes are combined in the table. Separation of the two green types in other cultures was less difficult, although it is apparent that some pale green plants have been included in the green class.

GOLDEN YELLOW (*y*)

The type known as golden yellow behaves as a monohybrid recessive as shown by data in table 15.

These golden yellow seedlings gradually develop chlorophyll and finally reach maturity, although growing much more slowly than their green sibs. These plants can, however, be distinguished in the mature stage, due both to size and to the peculiar distribution of the chlorophyll. They produce mature rosettes that show a mottling

of yellow and green through the leaves, which looks much like the plant disease known as 'mosaic,' or rosettes on which the central and thus younger leaves of the plant are a clear yellow. These yellow leaves later develop chlorophyll and become normally green.

It would appear from table 15 that the golden yellows would be homozygous recessives; but this is not the case, for the seedlings from selfed 'yellow center' and from 'mottled' plants show some of them to be heterozygotes. Only one plant has yet been found which was homozygous for yellow.

TABLE 15
MONOHYBRID SEGREGATION OF GOLDEN YELLOW IN THE PROGENY OF
GREEN PLANTS

Culture No. 1921	Progeny segregation of seedlings	
	Green	Yellow
177P ₁₃	10	3
177P ₁₆	12	3
177P ₁₇	278	84
177P ₃₈	13	5
177P ₄₀	15	3
177P ₇₈	36	10
177P ₁₂₄	23	6
Total	387	114
Calculated 3:1	375.75	125.25
Deviation	11.25 ± 6.54	

That there are other genes which also produce yellow seedlings is evident from table 16. The three plants P₃₃, 66, and 74 were green as seedlings and normal green in the mature stage. They apparently were heterozygous for two recessive genes which produced the same or a very similar type of yellow. The progeny of P₂₅ indicate still another type of yellow indistinguishable phenotypically from those already mentioned. Here the production of chlorophyll in the seedling stage is dependent on the simultaneous presence of two dominant genes, and the absence of either one results in a yellow type of seedling.

Trow (1916) reports a similar case of complementary recessive genes in the production of albino seedlings in *Senecio*, another genus of the Compositae.

TABLE 16
 SHOWING SEEDLING SEGREGATION IN THE PROGENY OF GREEN PLANTS INDICATING
 COMPLEMENTARY RECESSIVE GENES FOR GOLDEN YELLOW AND
 DUPLICATE GENES FOR CHLOROPHYLL

Culture No.	Progeny segregation of seedlings	
	Green	Yellow
21.177P ₃₉	44	3
21.177P ₆₆	13	1
22.177P ₇₆	45	3
Total	102	7
Calculated 15:1	102.19	6.81
Deviation	0.19 ± 1.70	
21.177P ₂₅	22	13
Calculated 9:7	19.687	15.312
Deviation	2.312 ± 1.98	

VIRESCENT YELLOW (v)

A third type of seedling called virescent yellow has a small amount of green color in addition to the yellow. These seedlings, like the yellow ones, may produce two types of mature plants, namely, pure green plants and green plants with pale green younger leaves at the center of the rosette. The data at present indicate that virescent plants are produced when a gene dominant to yellow but recessive to green is present with the gene for yellow, which changes yellow seedlings to virescent and yellow-center rosettes to pale green centers. When virescent plants are selfed, then green, virescent, and yellow are obtained, but no virescent plants have appeared in the progeny of yellow plants.

It is hoped that in another place it will be possible to publish more extensive data and a complete discussion of the inheritance of chlorophyll deficient characters in *Crepis* which cannot be given at this time.

GENERAL DISCUSSION

In order to establish and preserve true breeding strains of the different types observed in the cultures, type plants were self-pollinated in successive generations. This most intense type of inbreeding affected these cultures in very much the same way as inbreeding has affected maize. Reduction in size and a slower rate of growth were the most noticeable results of inbreeding together with a slight increase in sterility. Most of the experiments to show the effect of inbreeding in plants have been with domesticated forms in which it is possible to have a genotypic constitution that might not exist in a wild state, because characteristics which would unfit the individual for survival in natural conditions are often preserved under the artificial conditions of cultivation. The inference is that wild species would differ in fewer genes than their cultivated relatives. However, the inbreeding experiments on *Drosophila* (Castle, 1906) produced no bad effects. Collins (1919) states that self-fertilization in teosinte, a wild relative of maize, causes no loss of vigor such as is known to occur in maize. On the other hand, Darwin (1876) concluded that wild species which are naturally cross-pollinated are, on the whole, adversely affected by inbreeding. It appears then that the results of inbreeding any race, cultivated or wild, would be an index to its genotypic heterozygosity or homozygosity. With this as a criterion, there is indicated a condition of germinal heterozygosity in *Crepis capillaris*. There appears to be a certain similarity between wild heterozygous species of *Crepis* and the cultivated races of maize in the type of recessive genes which persist in the genotype. In maize, a number of genes are present which produce characters that are so abnormal (sterility, extreme dwarfs, albinos) that they are propagated only with difficulty and would seldom be found under natural conditions. Examples of similar forms have appeared in inbred strains of *Crepis*. It may therefore be considered that natural selection has not eliminated these genes from the germinal material of the wild species. The genes in *Crepis* which affect vigor also produce results comparable to similarly acting genes in maize.

Evidence of the genotypic heterozygosity of *capillaris* has also been gained from another source. Seeds have been obtained from widely separated localities and grown side by side in the greenhouse

and garden. The number of different forms resulting either in the first or later generations and as a result of controlled cross-pollinations show that the germinal material was indeed far from homozygous. It is of importance, because of some current theories regarding the influence of the habitat upon the genotype of a local species (Turesson, 1922), to observe the behavior of these various forms when grown in as nearly identical conditions as can ordinarily be furnished in a greenhouse or garden. Plants belonging to many different genera were collected by Turesson from contrasted habitat localities in Sweden and grown together in a common garden. He found that in general each particular type of a species found in each of several different habitats maintained its characteristics in the absence of the habitat to which it seemed especially modified. He sees in such phenomena a refutation of the theory, now generally held, that the form predominating in a given locality occurred as a chance mutation or recombination and was preserved through natural selection. The theory substituted for this is Lamarekianism expressed in modern terminology, namely, habitat causes a change in the fundamental genotype of the species such that a phenotype is developed which permits the plant to flourish in a specialized habitat. His report deals principally with three types of plants in all his species, viz., dwarf forms, upright or erect forms, and spreading or procumbent forms, each of which was found in a location favorable to the existence of that type while unfavorable to the other types; and each thus becomes a demonstration of the effects of natural selection. In our study of *Crepis* forms we have not been fortunate enough to study wild populations of *Crepis* in all of the localities from which we have obtained seed, but we have produced hereditary strains of erect forms, spreading forms, and dwarf forms from the same habitat at Eureka, a fact which does not especially favor the existence of any one type. Dwarf forms have also appeared in cultures from other places (France and Denmark), whose definite habitat characteristics are unknown to us. Similar plant forms are well known to occur sporadically in many wild and domesticated species. Mutations giving rise to prostrate and dwarf types in plants are not infrequent when compared to other types of change. If we accept the idea of a *genotypic response* of the species to the habitat, are we not also admitting the inconstancy of the gene, a theory which is no longer tenable? Continuing the assumption, it is not clear why these different hereditary types, such as we have in *Crepis*, remain constant in a single unvarying habitat.

The very fact that they do not approach a common type under cultivated conditions supports the theory of the constancy of the gene and is evidence of the inability of the habitat to induce genotypic changes.

The occurrence of duplicate genes in other plants has brought forth the opinion that they may indicate the presence of duplicated chromosomes. Three cases of duplicate genes have been found in *Bursa* (Shull, 1920), a plant having 32 chromosomes (4×8), while a case of triplicate genes is reported in a wheat (Nilsson-Ehle, 1909) which has 42 chromosomes. This number is three times the number (14) found in several species of *Triticum* (Sax, 1921). Several pairs of duplicate genes have been found in *Crepis capillaris*. No plants producing such ratios have been examined cytologically, but in no visible way do they differ from plants which give 3 to 1 ratios for the same characters. From what is known regarding the effect of duplication of single chromosomes or of whole sets of chromosomes in *Datura* (Blakeslee, 1922) and in *Nicotiana* (Clausen and Goodspeed, 1924), it is difficult to suppose duplication of chromosomes has occurred here. That we have parallel mutations in identical loci of two chromosomes of the same kind derived from a form with a different number by some meiotic irregularity is equally improbable, for *capillaris* has but three pairs of chromosomes, no two similar enough in size to be construed as duplicates. There are several other ways to account for the appearance of duplicate genes, some of which have been discussed by Shull (1918). Four of these possibilities are (a) the occurrence of similar gene mutations in different chromosome pairs; (b) the mating of non-homologous chromosomes; (c) duplication of entire chromosomes; and (d) duplication of sections of chromosomes. The possibility of a chromosomal duplication as the cause of the origin of duplicate genes in *Crepis* is very unlikely, as has been shown above. The other possibilities cannot be dealt with so readily. It would appear, however, that, had duplication of a section of a chromosome taken place, other characters, the genes for which were located in the duplicated section, should show similar inheritance ratios. As a matter of fact, two other characters in *Crepis capillaris* give ratios of 15 to 1, but in the one case tested (revolute \times smooth ribs) the type of linkage demanded by such an hypothesis was not obtained. Mating of non-homologous chromosomes should also result in duplication of other genes which should show linkage relations. Although only a small amount of critical data is as yet available, no confirmation of the linkage relations demanded

by these two methods of gene duplication has been obtained. Shall rejected the idea of the occurrence of two independent mutations as a cause of duplication of genes in *Bursa* on the ground that the characters were of such a complex nature that the occurrence of two independent mutations producing identically the same somatic results was on the verge of impossibility. The characters in *Crepis* for which there are duplicate genes cannot be considered as complex, and the occurrence of similar mutations in non-homologous chromosomes therefore seems at the present time to be the more reasonable explanation of the origin of duplicate genes in this species.

Sturtevant (1921) has shown that some points in the germinal material of a given species are more susceptible to mutations than others. There is evidence that such a mutating locus occurs in *capillaris*, for the same character, bald, has appeared in a number of strains derived from widely separated localities. The identity of these genes for bald has been proved in all cases except one (France) by crosses in which they proved to be allelomorphie. That a certain locus may mutate in the same way in other species is at least indicated by the fact that this character is now known to occur in four other species, none of which has been grown extensively among our cultures. The gene for bald is recessive in *capillaris* and is also recessive in the species cross, *setosa* \times *capillaris*.

No less interesting and unique is the group of complementary genes found in *C. capillaris* where the appearance of three such pairs of genes are concerned with the inheritance of leaf characters and a fourth with chlorophyll. It is not strange, however, that a greater number of complex gene relations should be encountered in a species containing a low number of chromosome pairs than in species having a larger number, unless the larger number results from reduplication. There is probably a minimum number of genes which is necessary in any species, and there is no reason to believe, a priori, that a species with a larger number of chromosomes need have a correspondingly larger number of genes. There is also evidence from *Drosophila* that the genes are distributed at random in each chromosome (except in cases of multiple allelomorphs) and among the chromosomes. When this basic number of genes is distributed among a large number of chromosomes, more characters will show simple types of inheritance. When this basic number is distributed in a fewer number of chromosomes, there will necessarily result more complex types of inheritance.

SUMMARY

1. Plants of *Crepis capillaris* are largely cross-fertilized, and this mode of reproduction operates to maintain a condition of genotypic heterozygosity.

2. Inbreeding wild plants thus produced results in the production of a number of pure races which show loss of vigor and reduction in size similar to the effects produced by inbreeding maize.

3. Four sets of duplicate genes are found to be responsible for the inheritance of four different characters. Two of these characters are shown not to be linked. Duplicated genes do not indicate duplicated chromosomes, for each pair is morphologically different from the others.

4. The recessive character 'bald' has appeared in a number of unrelated strains. This is evidence that a certain locus in one chromosome pair mutates more frequently in the same way than do other loci. The appearance of bald in other species may be due to a similar gene in each of these four species.

5. Several types of chlorophyll variations have appeared. Some show monohybrid recessive relations when contrasted with the normal condition, while others show more complex relations.

6. The different forms from widely separated localities show no tendency to approach a common type when grown continuously in the same place.

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EXPLANATION OF PLATES

PLATE 45

Fig. 1. A rosette of the *viridis* race on the left with a pallid rosette on the right.

Fig. 2. A typical rosette of the *scalaris* H6 race, showing blunt lobes, ruffled wing on midrib, constricted base of lateral lobes, and a twisting of the lateral lobes.

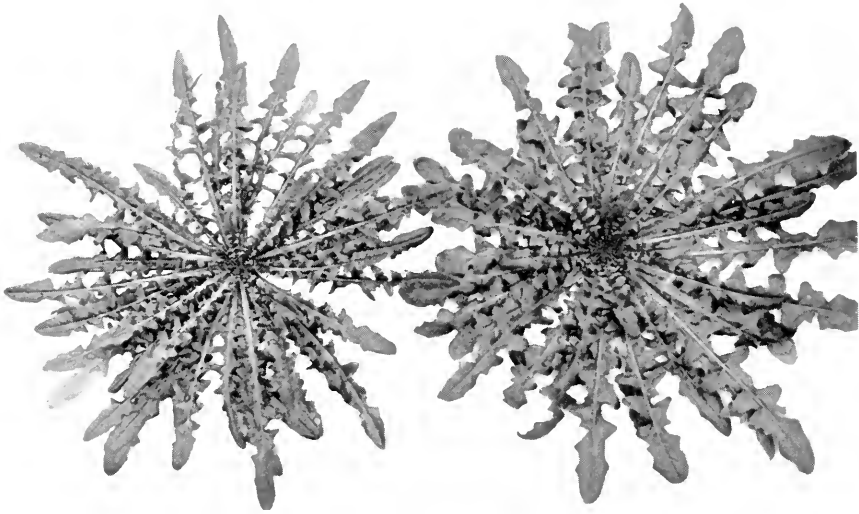


Fig. 1

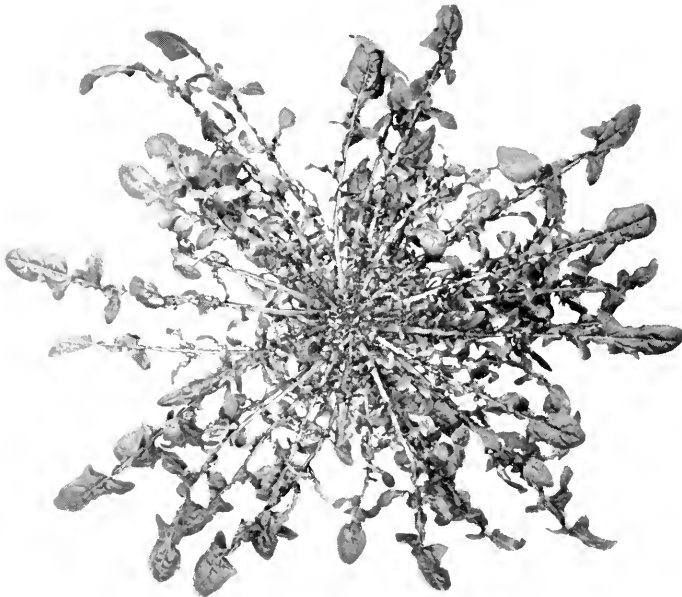


Fig. 2

PLATE 46

Fig. 1. A rosette of simplex Z9 on the left, and at the right the aberrant pinnatifid type which appears in all cultures.

Fig. 2. A rosette of the scalaris e29 race.



Fig. 1



22-16

Fig. 2

PLATE 47

Fig. 1. A typical rosette of the pinnatifid leaf, *scalaris* e28.

Fig. 2. A rosette showing revolute leaves.



Fig. 1

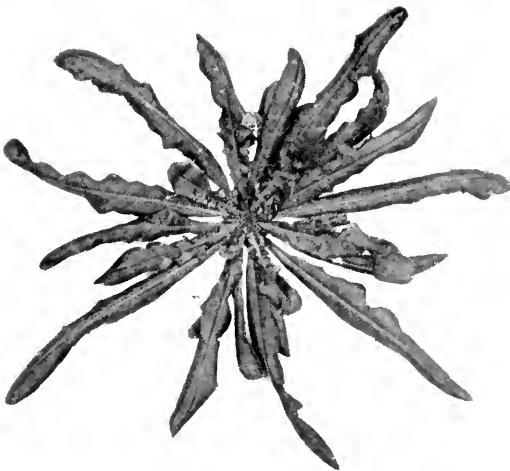


Fig. 2

PLATE 48

Fig. 1. The bicephalic type of fasciation.

Fig. 2. A mature dwarf II plant.



Fig. 1



Fig. 2

PLATE 49

Fig. 1. Two dwarf III plants with two normal sibs.

Fig. 2. A typical plant from the race with the spreading habit.

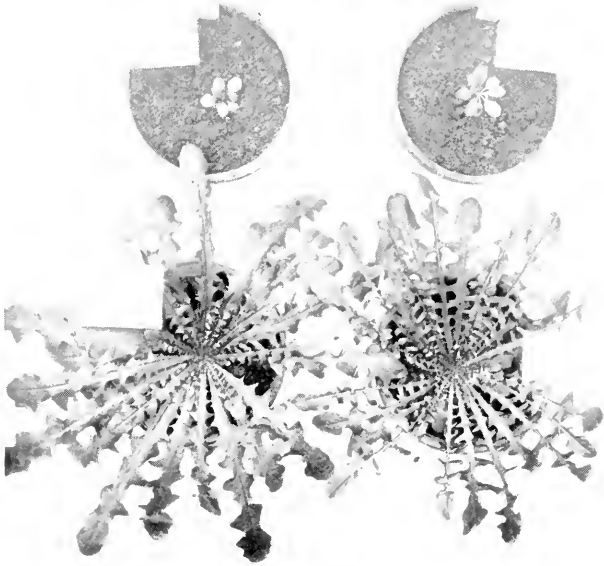


Fig. 1



Fig. 2

PLATE 50

Fig. 1. A typical plant of the erect growth habit.



PLATE 51

- Fig. 1. Palea on the left with a receptacle of a normal plant on the right.
Fig. 2. Three F_2 rosettes from the cross, scalaris \times simplex.



Fig. 1

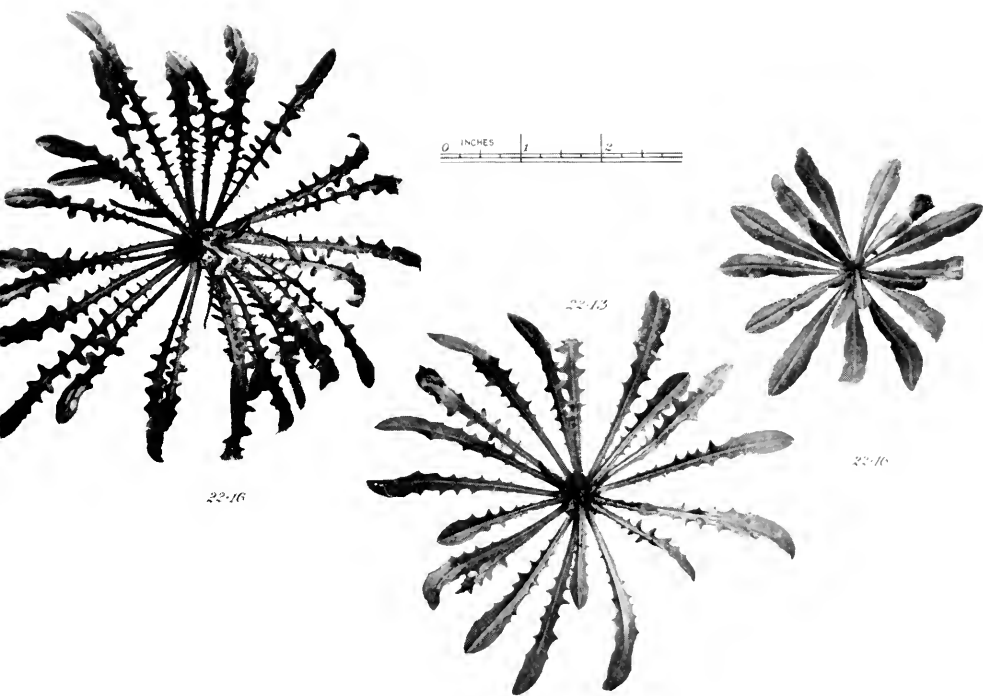
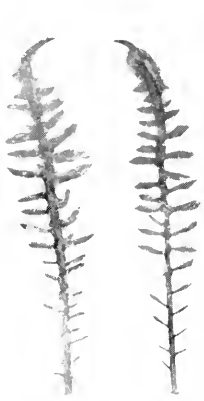


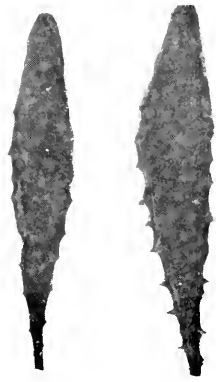
Fig. 2

PLATE 52

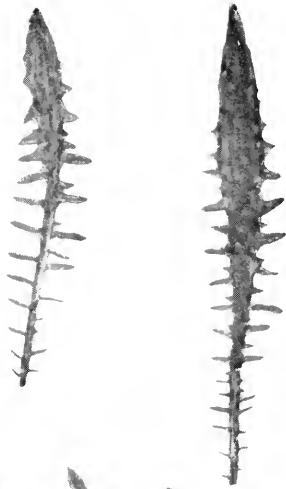
Fig. 1. Typical leaves from two plants of each of the parent strains and of the F_1 , together with one leaf from each of eight F_2 plants, which show the results obtained when scalaris and simplex plants are crossed. Note the appearance in F_2 of the curved terminal lobe typical of the scalaris grandparent.



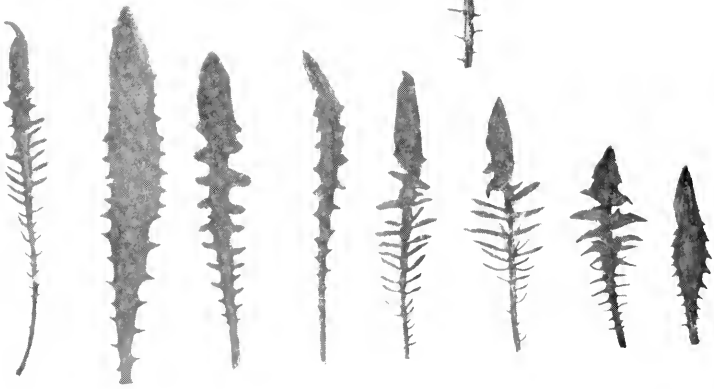
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F₁



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March 5, 1925

CHROMOSOME NUMBER AND INDIVIDUALITY
IN THE GENUS CREPIS

I. A COMPARATIVE STUDY OF THE CHROMOSOME
NUMBER AND DIMENSIONS OF NINETEEN SPECIES

BY

MARGARET CAMPBELL MANN

UNIVERSITY OF CALIFORNIA PRESS
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CHROMOSOME NUMBER AND INDIVIDUALITY
IN THE GENUS *CREPIS*

I. A COMPARATIVE STUDY OF THE CHROMOSOME
NUMBER AND DIMENSIONS OF NINETEEN SPECIES

BY

MARGARET CAMPBELL MANN

(Contribution from the Division of Genetics, University of California)

Because most of the species of the genus *Crepis* have low chromosome numbers, it offers obvious advantages for the study of comparative chromosome relations. The chromosome individuality of certain species is very distinct, so much so that it could be used as a diagnostic character in specific determination. These facts lead to an inquiry to discover first, whether upon careful analysis all species would prove to differ in chromosome individuality, and second, what relations the chromosome groupings of different species bear to one another. This question has been previously touched upon in several papers by Rosenberg (1909, 1918, 1920) and in a recent contribution by Marchal (1920). Rosenberg (1918) called attention to the fact that the genus *Crepis* possesses a great variety of chromosome numbers. His summary showed species with 3, 4, 5, 8, 9, and 20 pairs. In order to determine how such numerical differences had arisen within the genus, he measured the chromosomes of a three and a four-pair species, *capillaris* (*Reuteriana* of Rosenberg) and *tectorum*, respectively, and found, on the basis of measurements of homotypic anaphase chromosomes, that three of the chromosomes of the two species corresponded accurately in size and that the fourth pair of *tectorum* averaged slightly shorter than the shortest of *capillaris*. He noted that the two shortest chromosomes of *capillaris* often mate later than the other two in p. m. c. and finds associated with this fact a tendency toward lagging and irregular division. From these data he

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concluded that the four-pair species have arisen from a three-pair species by the fusion of two gametes each of which has received an extra short chromosome. Although he did not publish measurements on the two five-pair species which he studied (*rubra* and *multicaulis*), he believed that both have three of the short chromosomes, and that these types have originated by a repetition of the process which gave rise to the four-pair types. In his 1920 contribution he changes his count in *biennis* from twenty to twenty-one pairs and concludes that it represents the three chromosomes of *capillaris* multiplied fourteen times.

Marchal, whose work was done without knowledge of Rosenberg's paper, expressed (1920) the belief that four is the ground number of the genus *Crepis*. He noted that p.m.c. of a slightly aberrant *capillaris* plant had what appeared to be a large quadrivalent multiple chromosome plus two smaller but equal elements, and that most of the species of *Crepis* seemed to have four pairs of chromosomes. He therefore concluded that *capillaris* had arisen from the type by end-to-end union between two chromosomes. He believed that the differences in length which had been noted for *C. lanceolata platyphylla* (Tabara and Ishikawa, 1911) could be accounted for by bipartition of one chromosome of a species with four pairs. He further suggested that six-pair species might arise by doubling of the three, and an eight-pair species by doubling of the four. He counted sixteen pairs for *biennis* and noted that, while the individual chromosomes in the p.m.c. of this species appeared somewhat smaller than those of certain four-chromosome species, the total mass was much greater. He then concluded that *biennis* is an eight-ploid species.

MATERIAL AND METHODS

A large number of species of the genus *Crepis* have been grown and identified in the greenhouse of the Division of Genetics of the University of California by Professor E. B. Babcock, thus making it possible to be certain of the specific determination of the material which was studied cytologically. Since the chromosome numbers which have been found to characterize the species thus identified differ in several instances from previously published counts, the data are presented in a convenient form in table 1. The root tips were fixed in chrom-acetic-urea and stained in Heidenhain's iron-haematoxylin. In most species the reduced number has also been counted by Belling's iron-aceto-carmin method.

TABLE 1
CHROMOSOME COUNTS OF 27 SPECIES OF CREPIS

Species	Number		Author
	N	2N	
<i>alpina</i> L.....	4		Marchal (1920)*
		10	Rosenberg (1920)†
	5	10	Mann (1922)‡
<i>amplexifolia</i> Willk.....	4	8	Mann
<i>aspera</i> L.....	4		Marchal (1920)
	4	8	Mann (1922)
<i>aurea</i> (L.) Reichb.....	5	10	Mann
<i>biennis</i> L.....	16		Marchal (1920)
	20		Rosenberg (1918)
	21		Rosenberg (1920)
	20	40	Mann (1922)
<i>blattarioides</i> Vill.....	4		Marchal (1920)
		8	Rosenberg (1920)
	4	8	Mann
<i>breviflora</i> Delile.....	4	8	Mann
<i>bulbosa</i> (L.) Tausch.....	9	18	Mann
<i>bursifolia</i> L.....	4	8	Mann
<i>capillaris</i> (L.) Wallr.....	3	6	Rosenberg (1909), Mann (1922)
<i>dioscoridis</i> L.....	4		Marchal (1920)
	4	8	Mann (1922)
<i>foetida</i> L.....	4		Marchal (1920)
	4	8	Rosenberg (1918)
	5	10	Mann (1922)
<i>grandiflora</i> Tausch.....	4	8	Mann
<i>incarnata</i> Tausch.....	4	8	Mann
<i>japonica</i> (L.) Benth.....	8	16	Tahara (1910), Mann (1922)
<i>myriocephala</i> Coss. et D. R.....	4	8	Mann (1922)

* Marchal gives 1914 as the date of his counts, but they were not published until 1920.

† Figured but not mentioned in the text.

‡ Cited from Report of the College of Agriculture, University of California, July 1, 1921-June 30 1922.

TABLE 1—(Continued)

Species	Number		Author
	N	2N	
<i>neglecta</i> L.....	4	8	Rosenberg (1918), Mann (1922)
<i>palestina</i> Boiss. Bornmüller.....	4	8	Mann
<i>parviflora</i> Desf.....	4	8	Rosenberg (1918), Mann (1922)
<i>pulchra</i> L.....	4	8	Rosenberg (1920), Mann (1922)
<i>rubra</i> L.....	4	10	Marchal (1920)
	5		Rosenberg (1918), Mann (1922)
<i>setosa</i> Hall.....	4	8	Mann (1922)
<i>sibirica</i> L.....	4	10	Marchal (1920)
	5		Mann (1922)
<i>Sieberi</i> Boiss.....	6	12	Mann (1922)
<i>taraxacifolia</i> Thuill.....	6	12	Beer (1912)
	4	8	Digby (1914), Mann (1922)
<i>tectorum</i> L.....	4	8	Juel (1905), Mann (1922)
<i>vesicaria</i> L.....	4	8	Mann

Table 1 shows that, while four is the most common haploid number for the twenty species studied, five is also fairly frequent. The other numbers (3, 6, 8, 9, and 20) are each represented by a single species. It is obvious that chromosome measurement should show whether cross-division, union into multiples, addition by non-disjunction, or combinations of these methods are sufficient to account for the differences in number found in the genus. It is also possible that hybridization between species with different chromosome numbers might account for the origin of certain cytological peculiarities.

For some species the cytological material is far more abundant than it is for others, so that it is possible to measure only somatic metaphases in which all the chromosomes are fairly straight. The tendency of the long chromosomes of *Crepis* to twist is a source of considerable error where relatively poor material is available. The finest metaphase figures are to be found in the upper portion of the rapidly growing region of the root in seedlings, and in roots from adult plants. The region containing fine figures is greater in roots from the latter than

in the short root of the cotyledon stage, because there is a longer growing area in which the cytoplasm is less dense than it is at the tip, so that the chromosomes spread out more freely and the picture is less obscured by cytoplasmic inclusions.

Table 3 is a compilation of measurement data for somatic metaphase figures in nineteen species of *Crepis*. In each case, except *japonica* and *sieberi*, ten somatic polar metaphases were drawn with a camera lucida. The magnification of the drawings is 4000 diameters. A moistened thread was placed along the center of the drawing of each chromosome, and then straightened and measured in millimeters. The figures were then placed in columns, the two largest in the first, and so on down to the two smallest. A sample of these records for a five-pair species, *alpina*, is given below in table 2.

TABLE 2

ACTUAL MEASUREMENTS OF DRAWINGS					DIFFERENCES FROM AVERAGE					
1	2	3	4	5	Total Length	1	2	3	4	5
32 mm.	25 mm.	14 mm.	13.5mm.	13mm.	195 mm.	+5.8	+5.7	-0.5	+0.4	+0.8
31	27	14	13	12.5						
22.5	20	15.5	13	11.5	163 mm.	-1.7	-1.3	+1.0	-0.1	-0.7
24.5	18	14.5	13	11						
30.5	21	17	14.5	12.5	179 mm.	+4.3	-0.3	+2.5	+1.4	+0.8
22	19	15	14.5	13						
21.5	17	13	12	10.5	153 mm.	-2.7	-2.3	-1.5	-1.1	-0.7
23.5	19	13	12	11.5						
23	21.5	16.5	14	12	174 mm.	+2.8	+0.2	+2.0	+0.9	-0.2
29	20	15	12	11.5						

It is evident that even measurement by the rather crude method described above gives a fairly definite clue to the individuality of the species. It will also be noted that when the larger figure of each set is compared with the average for the chromosome, obtained by dividing the sum of the ten larger of the twenty chromosomes of one type by ten, the deviations for any one metaphase set are generally in the same direction (+ or -). (See column headed "Differences from the average.") This deviation indicates that the error of measurement was not sufficient to conceal the fact that the chromosome lengths of a species maintain certain size relations at least throughout the later periods of shortening. It also shows that it is fair to use an average

so obtained in a comparative study like this. The larger figure of each set was considered the more accurate measurement and hence was used to secure the 'corrected' totals and averages which appear in table 3.

TABLE 3
MEASUREMENT DATA FOR NINETEEN SPECIES OF CREPIS

Species	Haploid chromosome number	Corrected average total length	Corrected average for individual chromosomes										
<i>C. capillaris</i>	3	61.4	26.2	20.4	14.8								
<i>C. neglecta</i>	4	61.7	24.5	16.2	11.2	9.8							
<i>C. setosa</i>	4	63.2	22.3	17.8	14.0	9.1							
<i>C. parviflora</i>	4	69.9	25.3	20.5	14.4	9.7							
<i>C. bursifolia</i>	4	78.5	24.3	22.0	19.5	12.7							
<i>C. aurea</i>	5	83.5	21.0	18.0	16.2	15.1	13.2						
<i>C. aspera</i>	4	82.6	23.9	21.5	19.7	17.5							
<i>C. alpina</i>	5	87.3	26.2	21.3	14.5	13.1	12.2						
<i>C. taraxacifolia</i>	4	88.4	26.1	23.3	21.2	17.8							
<i>C. tectorum</i>	4	88.7	28.1	23.2	20.2	17.2							
<i>C. blattarioides</i>	4	91.1	29.0	23.8	20.6	17.7							
<i>C. japonica</i> ^a	8	92.6	15.7	13.5	12.2	11.5	10.8	10.0	9.7	9.2			
<i>C. foetida</i>	5	93.7	25.0	20.8	17.7	15.8	14.4						
<i>C. bulbosa</i>	9	100.5	13.9	12.8	12.1	11.7	11.1	10.6	10.1	9.6	8.6		
<i>C. rubra</i>	5	102.9	29.4	23.9	18.5	16.2	14.9						
<i>C. dioscoridis</i>	4	109.4	35.9	29.3	24.9	19.3							
<i>C. sicberi</i> ^a	6	109.6	26.8	21.4	17.7	16.0	15.2	12.5					
<i>C. pulchra</i>	4	112.1	36.7	30.6	25.5	19.3							
<i>C. sibirica</i>	5	143.6	41.9	32.4	27.6	23.2	18.5						

^a Averages from less than ten figures.

The reliability of such measurements and the evidence for the constancy of specific individuality have been further corroborated by a study of chromosome measurements of the F_1 's of two species-hybrids, *setosa* × *tectorum* (fig. 1) and *setosa* × *dioscoridis* (fig. 2).¹ It will be noted from table 3 that all three species involved have four pairs and that the chromosome sizes are far more different in the two latter than in the two former species. In both F_1 's, however, it was possible to determine the source of the chromosomes by means of measurement data, and this was facilitated by the peculiar semidetached tip of the longest chromosome of *setosa* (fig. 3), by which it may usually be identified. Since only one member of a set is present in each F_1 figure, it seemed best to compare the averages for the F_1 's with the uncorrected averages for the species involved. The results are tabulated below:

¹ For the use of these hybrids and the data on hybridization given below, I am indebted to Dr. J. L. Collins of this laboratory.

TABLE 4

<i>setosa</i> × <i>dioscoridis</i>	39.9	33.6	28.9	23.1	22.1	18.1	13.7	10.3
<i>setosa</i>					22.3	17.8	14.0	9.1
<i>dioscoridis</i>	34.2	28.9	24.9	20.6				
	+5.7	+4.7	+4.0	+2.5	-0.2	+0.3	-0.3	+1.2
<i>setosa</i> × <i>tectorum</i>	29.4	24.1	21.2	16.8	21.0	18.9	13.3	8.9
<i>setosa</i>					22.3	17.8	14.0	9.1
<i>tectorum</i>	28.1	23.2	20.2	17.2				
	+1.3	+0.9	+1.0	-0.4	-1.3	+1.1	-0.7	-0.2

The important point is that one can identify the chromosomes of *dioscoridis* and of *tectorum* by measurement when they are in combination with those of *setosa* in an F₁ hybrid, so that it is evident that the specific differences in length noted are not the product of interaction between a certain cytoplasm and its chromosomes.

Since abundant material was available for *capillaris* (fig. 6), the first measurements, which were made on ten figures about as good as the average for all species, were checked by the use, first, of a mixture of slightly different metaphase stages (beginning to almost complete division) from a very short region of a single root tip, and, second, of a mixture from undivided figures from two different roots. These measurements show that averages for one chromosome in three different sets of ten from the same species may differ by as much as 3.55 mm., but that the averages give, in each case, very nearly the same differences between the lengths of the different pairs.

COMPARISON OF SPECIES

Crepis neglecta (fig. 7) has a very characteristic individuality, two of the pairs being very similar and distinctly shorter than any of the chromosomes of *capillaris*. Its total length is very similar to that of *capillaris*, so much so that one is inclined to test the cross-division hypothesis for this species. If the two shortest averages are added, their sum is practically the same as the average for the intermediate chromosome of *capillaris* and the other average lengths are very similar.

<i>capillaris</i>	26.2	20.4	14.8
<i>neglecta</i>	24.5	11.2+9.8=21.0	16.2
	-1.7	+0.6	+1.4

Attempts to cross the two species have as yet been unsuccessful.

Setosa (fig. 3), like *neglecta*, differs little from *capillaris* in total length. It contains, however, only one pair of chromosomes shorter than any in *capillaris*; otherwise it is rather similar to it.

<i>capillaris</i>	26.2	20.4	14.8	
<i>setosa</i>	22.3	17.8	14.0	9.1
	-3.9	-2.6	-0.8	+9.1

It has already been noted that the longest chromosome of *setosa* has a semidetached tip by which it may be recognized. This tip is usually at an angle to the main portion of the chromosome. In the figures given above the longest chromosome of *setosa* appears to have lost a portion of its length, while another pair of chromosomes averaging about ten units has been added. It is also possible that the longest chromosome has cross-divided, and that the peculiar chromosome of *setosa* really corresponds to the intermediate of *capillaris*.

<i>capillaris</i>	26.2	20.4	14.8	
<i>setosa</i>	17.8+9.1=26.9	22.3	14.0	
	+0.7	+1.9	-0.8	

If either of these possibilities represented the whole truth concerning the difference between the two species, we should expect reduction to be fairly normal following hybridization. As a matter of fact, *no pairing occurs* in the F_1 *setosa* (N=4) \times *capillaris* (N=3) (Collins and Mann, 1923), and as a consequence gametes are formed with 3, 4, and 6 chromosomes as shown by five plants (backcrosses to *setosa*), which have 7, 8, and 10 somatic chromosomes. It seems possible that new types differing in number and combination of chromosomes may be obtained by selfing such plants as the backcrosses with ten chromosomes.

Crepis parviflora (fig. 8) has a chromosome individuality much like that of *setosa*; the longer chromosome, however, averages slightly longer and does not appear to have a semidetached tip.

<i>setosa</i>	22.3	17.8	14.0	9.0
<i>parviflora</i>	25.3	20.5	14.4	9.7
	+3.0	+2.7	+0.4	+0.7

It is evident that *parviflora* is more similar to *capillaris* than *setosa*, but like *setosa* it has an additional short pair of chromosomes.

<i>capillaris</i>	26.2	20.4	14.8	
<i>parviflora</i>	25.3	20.5	14.4	9.7
	-0.9	+0.1	-0.4	+9.7

The first hypothesis for *setosa* appears to be the more probable for *parviflora*. If it were true, one would have to account for the additional chromosome of 9.7 units by hybridization between two such forms as

neglecta and *capillaris*. The hybridization results for *setosa* × *capillaris* given above indicate that new types with new combinations of chromosomes may arise in this manner. It will be interesting to observe the results of crossing *setosa* and *parviflora*.

Bursifolia (fig. 9) appears to have an extra element of the size of the intermediate chromosome of the *capillaris* series:

<i>capillaris</i>	26.2		20.4	14.8
<i>bursifolia</i>	24.3	$\frac{22+19.5}{2}=20.7$		12.7
	-1.9		+0.3	-2.1

It's average total length is 17.1 units longer than that of *capillaris*.

Crepis taraxacifolia (fig. 10), *tectorum* (fig. 5), and *blattarioides* (fig. 11) have very similar chromosome groups.

<i>taraxacifolia</i>	26.1	23.3	21.2	17.8
<i>blattarioides</i>	29.0	23.8	20.6	17.7
<i>tectorum</i>	28.1	23.2	20.2	17.2

All the chromosomes of these three species tend to average slightly larger than those of *capillaris*, but the differences do not greatly exceed those of the different averages for *capillaris*. If we suppose that the intermediate chromosome of *capillaris* has been duplicated in this group of species, the correspondence is somewhat bettered.

Average of <i>taraxacifolia</i> , <i>tectorum</i> , and <i>blattarioides</i>	27.7	22.05	17.6
Average of <i>capillaris</i>	26.2	20.40	14.8
	+1.5	+1.65	+2.8

It is obvious that the relative lengths of the chromosomes in these three species are very similar to those in *capillaris*.

Tectorum and *capillaris* were repeatedly crossed by Collins (1920), but the F₁ developed only as far as the cotyledon stage. This indicates an incompatibility of the chromosomes or cytoplasm hard to account for on the basis of mere addition of similar material, especially when one considers that trisomic forms which come to maturity appear to be not uncommon among plants and animals. It will be very interesting to know whether others of the group of species indicated above will behave like *tectorum* in crosses with *capillaris*, and whether they will intercross.

Aspera (fig. 12) is like the group discussed above except that the longest chromosome appears to be rather short.

<i>capillaris</i>	26.2		20.4	14.8
<i>aspera</i>	23.9	$\frac{21.5+19.7}{2}=20.6$		17.5
	-2.3		+0.2	+2.7

Crepis bursifolia, *taraxacifolia*, *tectorum*, *blattarioides*, and *aspera* might all be derived from *capillaris* by duplication of the intermediate pair of chromosomes.

The five-pair species listed below, although generally rather similar in chromosome individuality, show certain distinct differences.

						Total length
<i>aurea</i>	21.0	18.0	16.2	15.1	13.2	161.9
<i>alpina</i>	26.2	21.3	14.5	13.1	12.2	174.6
<i>foetida</i>	25.0	20.8	17.7	15.8	14.4	187.4
<i>rubra</i>	29.4	23.9	18.5	16.2	14.9	205.8

Aurea (fig. 13) is outstanding since it lacks a long chromosome of about twenty-five units. The figures are excellent, so that the averages must be considered as very nearly accurate. *Aurea* is also very distinctive morphologically. *Alpina* (fig. 14), *foetida* (fig. 15), and *rubra* (fig. 16) are much more alike in chromosome individuality. *Alpina* seems to have three pairs resembling the shortest chromosome of *capillaris*, and to be cytologically very like it otherwise.

<i>capillaris</i>	26.2	20.4		14.8
<i>alpina</i>	26.2	21.3	$\frac{14.5+13.1+12.2}{3}$	= 13.2
	0	+0.9		-1.6

Foetida might also have three duplicates of the shortest chromosome of *capillaris*.

<i>capillaris</i>	26.2	20.4		14.8
<i>foetida</i>	25.0	20.8	$\frac{17.7+15.8+14.4}{3}$	= 15.9
	-1.2	+0.4		+1.1

The figures for *rubra* compare better with those of *capillaris* if we average the two intermediates and the two shortest together.

<i>capillaris</i>	26.2		20.4	14.8
<i>rubra</i>	29.4	$\frac{23.9+18.5}{2}$	= 21.2	$\frac{16.2+14.9}{2}$
	+3.2		+0.8	+0.7

It was noted above that Rosenberg (1918) suggested that probably the small chromosome of *capillaris* had been duplicated twice for *rubra*. It will be seen from the figures that duplication of the intermediate and of the short chromosome appears more probable on the basis of the measurements presented here.

Crepis japonica (N=8) (fig. 17) and *bulbosa* (N=9) (fig. 18) are rather similar in chromosome individuality, but are totally different from all the rest of the species studied in chromosome number and size.

<i>japonica</i>	15.7	13.5	12.2	11.5	10.8	10.0	9.7	9.2	
<i>bulbosa</i>	13.9	12.8	12.1	11.7	11.1	10.6	10.1	9.6	8.6

It is, of course, possible that *japonica* might have been derived from a species like *tectorum* by cross-division of every chromosome, or vice versa. When we test this hypothesis by adding the averages for the two largest, the next two, etc., of *japonica* together, the results are rather striking.

<i>japonica</i>	}	15.7	12.2	10.8	9.7
		<u>13.5</u>	<u>11.5</u>	<u>10.0</u>	<u>9.2</u>
		29.2	23.7	20.8	18.9
<i>tectorum</i>		28.1	23.2	20.2	17.2
		+1.1	+0.5	+0.6	+1.7

It is at least obvious that tetraploidy could not explain the chromosome individuality of *japonica* while cross-division might do so.

Crepis sieberi (fig. 19) is the only species so far studied which has six pairs of chromosomes. It looks as if it might have four pairs of short chromosomes:

<i>capillaris</i>	26.2	20.4		14.8
<i>sieberi</i>	26.8	21.4	$\frac{17.7+16+15.2+12.5}{4}$	=15.3
	+0.6	+1.0		+0.5

or two intermediate and three short pairs:

<i>capillaris</i>	26.2	20.4		14.8
<i>sieberi</i>	26.8	$\frac{21.4+17.7}{2}$	$\frac{16+15.2+12.5}{3}$	=14.6
	+0.6	-0.9		-0.2

Crepis pulchra (fig. 21) and *dioscoridis* (fig. 4) are very similar to one another in chromosome length.

<i>pulchra</i>	36.7	30.6	25.5	19.3
<i>dioscoridis</i>	<u>35.9</u>	<u>29.3</u>	<u>24.9</u>	<u>19.3</u>
Difference.....	0.8	1.3	0.6	0

C. sibirica (fig. 23), with five pairs, resembles *pulchra* and *dioscoridis* in chromosome measurements, and the average length of the two longest chromosomes, 36.5, indicates that it may have two instead of one of the longest type of chromosome.

<i>sibirica</i>	$\frac{41.9+32.4}{2}=37.1$	27.6	23.2	18.5
<i>dioscoridis</i>	35.9	29.3	24.9	19.3
Difference.....	1.2	1.7	1.7	0.8

If we suppose that this group of species has been derived from a type like *capillaris*, we must consider that the longest chromosome represents a multiple. If we subtract the intermediate average for *capillaris* (20.4) from the average of the longest chromosomes of all three species in this group (36.3), the remainder, 15.9, is only 1.1 units longer than the shortest chromosome of *capillaris*, indicating that an intermediate and a short chromosome might have united end to end to form an element averaging 36.3 units. Then if we average the two shortest chromosomes of these three species with the chromosome of 20.4 units, which, we have supposed has united with a short element, the average, 19.9, is so like the intermediate of *capillaris* as to suggest that it may have been duplicated in the group under consideration. When we look at the averages now, the figures compare very well.

<i>capillaris</i>		26.2	20.4	14.8
<i>pulchra, dioscoridis,</i> <i>and sibirica</i>	$\frac{30.6+29.3+27.6}{3}=29.1$	19.9	15.9	
		+2.9	-0.5	+1.1

These species obviously form a group by themselves, especially since it has been shown that the great size of the chromosomes in *dioscoridis* is maintained upon hybridization with a species like *setosa*.

DISCUSSION

For two reasons it is impossible to make any sweeping generalizations at this time concerning the data presented here. First, we do not yet know how *species* differing in chromosome number can arise, and second, we know too little about the genetics of *Crepis*. There are two known methods by which a single pair of chromosomes can be added to a complex, non-disjunction and species-hybridization, but in neither case has it been proved that *stable types* would ever result; and the formation of new species presupposes stability. It has been suggested that it is very improbable that stability is to be expected of tetrasomic individuals because the complex as a whole is unbalanced by the addition of chromosomes. This view seems to be borne out by observations on the cytology of tetrasomic plants of *Datura* (Belling and Blakeslee,

1924) and *Matthiola* (Frost and Mann, 1924). Both of these tetrasomic types are even feebler than the trisomic plants, and hence would have little chance of survival under unfavorable environmental conditions. The possibilities of species-hybridization as a source of differences in chromosome number within a genus are still less known. It might be argued with some plausibility that if a tetrasomic condition is unbalancing and associated with lessened viability, even less in the way of stability and viability should be expected of organisms having a pair of chromosomes from another species added to a complete specific complex. The *Drosophila* workers have found, however (Morgan, 1922), that a similar genic structure characterizes the chromosomes of several species of that genus, and if this is true of *Crepis*, one method may be as probable as the other. It has been shown (Collins and Mann, 1923) that new types with more chromosomes than either species possesses are formed when the F_1 *C. setosa* \times *C. capillaris* is backcrossed to *setosa*. It is only through further work on such types that the question of stability can be answered. The theoretical and practical value of such work is self-evident.

While the little work that has so far been done on tetrasomic plants tends to show that they would be expected to be somewhat unstable genetically, tetraploid plants, e. g., *Oenothera gigas*, breed true. That *Crepis biennis* may be an octaploid from a five-pair species is indicated by the following experimental evidence:

1. In the F_1 *C. setosa* \times *C. biennis* the twenty pairs of chromosomes from *biennis* form ten pairs.

2. In the backcross of this F_1 to *biennis* the thirty chromosomes from *C. biennis* form fifteen pairs.

The great size and vigor which distinguish it from the other species studied also indicate that it is polyploid. The evidence from chromosome measurements indicates strongly that *Crepis biennis* is the only one of the twenty species discussed in this paper that could owe its origin to polyploidy.

It would seem possible that, if the whole complex of one species were added to that of another by segregation following species-hybridization, zygotes formed by the union of two such gametes might be expected to give stable races differing in chromosome number from other species of the genus. There is no evidence that such a procedure has occurred in any of the species of *Crepis* discussed above.

There is at present little evidence that whole chromosomes can be lost and the resulting organisms be expected to give rise to new species. Genetical and cytological results on *Drosophila* (Bridges, 1921) indicate

that while 53 per cent of the expected flies lacking one of the small fourth chromosomes live, they are imperfect, weak, and often sterile. That a small portion of a chromosome may be lost or inactivated is indicated also by work on this fly (Bridges, 1919). Loss of this strain is attributed to the injurious effect of the deficiency upon viability, fertility, and productivity.

While loss of chromosomes appears to be somewhat improbable as a method by which one species can come to differ from another in chromosome number, the chromosome number of some species may be reduced as a result of permanent end-to-end union of certain chromosomes to form multiples. The differences in number noted for the *Acrididae* (McClung, 1917) appear to be of this type. One species, *Hesperotettix viridis*, shows considerable variation in chromosome union in different individuals, indicating that it may be in the process of producing new types of chromosome grouping. It is also decidedly variable morphologically.

There is some observational evidence that species differ from one another in chromosome number due to cross-division of all chromosomes of a complex. Marchal (1920), for example, reported that in the section Medium of *Campanula* the size of each chromosome of pollen mother cells is less when the haploid specific number is thirty-four than when it is seventeen.

It is difficult to understand how cross-division or union of chromosomes to form multiples could *cause* specific differences. In fact, a case from *Drosophila* reported by Mrs. Morgan (1922) indicates that while end-to-end union of the X-chromosomes may affect genetic results it has no effect upon specific characters. It seems simpler to suppose that such changes in chromosome complexes are the result rather than the cause of genetical differences between individuals, such as have been noted for *Hesperotettix viridis* and for the different species of the *Acrididae*.

In the genus *Drosophila*, it has been shown that chromosomes that look alike may carry very different genes. For example, in *D. willistoni*, Metz and Lancefield (1922) report that the X-chromosome is a V-shaped element similar to the second and third autosomes of *D. melanogaster*. Without this genetic evidence one would have said that these two species had the same type of chromosome complex. Such evidence is a timely warning to those who would draw hasty conclusions on the basis of data like those given above for *Crepis*. The genetical results from *Crepis* are still too scanty to permit of such tests.

SUMMARY AND CONCLUSIONS

1. With the exception of *neglecta* and possibly *setosa*, all the species of *Crepis* studied show significant increases in total length of the chromosome complex over that of *capillaris*, the single species with three pairs of chromosomes.

2. Generally speaking, increased number is associated with increased total length, but there are certain exceptions.

3. In so far as studies on chromosome individuality can determine, five of the species with four pairs of chromosomes might have two pairs like the intermediate chromosome of *capillaris*.

4. In *Crepis neglecta* (N=4) the two shortest chromosomes might have been derived by cross-division of a chromosome of the length of the intermediate chromosome of *capillaris*.

5. *Crepis setosa* (N=4) and *parviflora* (N=4) are very similar in total length and quite unlike all of the other species.

6. *Crepis dioscoridis* (N=4) and *pulchra* (N=4) have a long pair of chromosomes which is not represented in *capillaris* or in the other four chromosome species. It is possible that it might be a multiple chromosome. That this difference in length is not due to a difference in physiological condition or to error is shown by the fact that it is maintained when the *dioscoridis* chromosomes are in *setosa* cytoplasm in an F₁ between these two species. All the chromosomes of these two species can be distinguished in this F₁.

7. *Aurea* stands out among the species with five pairs because of its lack of an element like the longest chromosome of *capillaris*. The complexes of *rubra*, *foetida*, and *alpina* might all have been derived by duplication of certain chromosomes of *capillaris*. *Sibirica* seems to possess two chromosomes like the large element of *dioscoridis* and *pulchra*.

8. The single species with six pairs, *sieberi*, has chromosomes which are enough like those of *capillaris* in length to have been derived from it by chromosomal duplication. There appear to be but one pair of the large and the intermediate types, and four pairs like the short chromosomes.

9. *Japonica* with eight pairs might be derived by cross-division of all chromosomes of a species like *tectorum*.

10. *Bulbosa* (N=9) has short chromosomes like those of *japonica*.

11. *Biennis* ($N=20$) has chromosomes comparable in size to those of *capillaris*, and there is some experimental evidence which indicates that it is a polyploid from a five-pair species.

12. It is well understood that these data are simply suggestive, but it is hoped that they may be of some use in taxonomic and hybridization studies on *Crepis*. The evidence, based on especially favorable cytological material, shows that it is entirely unsafe to assume that even closely related species which have the same chromosome numbers are identical in chromosome individuality; or to assume polyploidy unless the sizes of the chromosomes have been compared.

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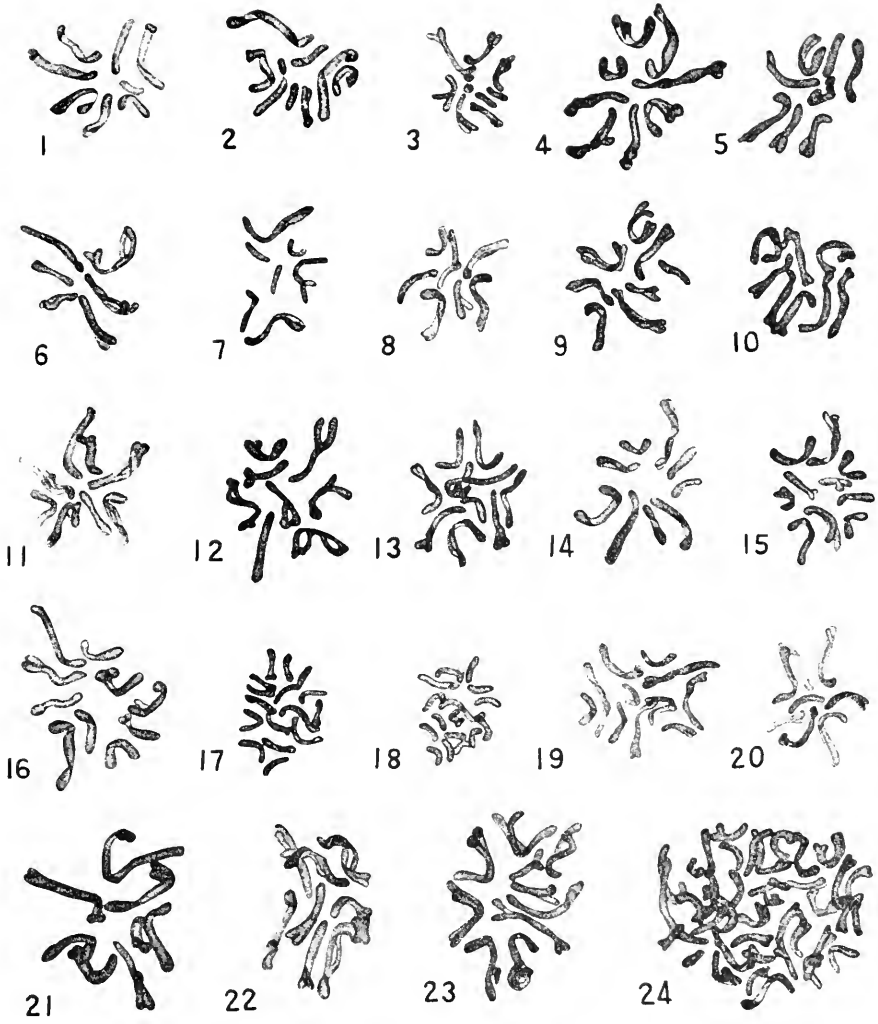
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PLATE 53

Somatic metaphases of *Crepis* species magnified 4000 diameters, using a B. and L. camera lucida mirror at 50, bar at 110, and a 1.8 mm. oil objective with an 18X Zeiss compensating ocular. Reduced in reproduction to 1800 diameters.

- | | |
|---|-------------------------|
| 1. <i>F</i> ₁ <i>setosa</i> × <i>tectorum</i> | 13. <i>aurca</i> |
| 2. <i>F</i> ₁ <i>setosa</i> × <i>dioscoridis</i> | 14. <i>alpina</i> |
| 3. <i>setosa</i> | 15. <i>foetida</i> |
| 4. <i>dioscoridis</i> | 16. <i>rubra</i> |
| 5. <i>tectorum</i> | 17. <i>japonica</i> |
| 6. <i>capillaris</i> | 18. <i>bulbosa</i> |
| 7. <i>neglecta</i> | 19. <i>siberi</i> |
| 8. <i>parviflora</i> | 20. <i>amplexifolia</i> |
| 9. <i>bursifolia</i> | 21. <i>pulchra</i> |
| 10. <i>taraxacifolia</i> | 22. <i>grandifolia</i> |
| 11. <i>blattarioides</i> | 23. <i>sibirica</i> |
| 12. <i>aspera</i> | 24. <i>biennis</i> |



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CHROMOSOME NUMBER AND INDIVIDUALITY
IN THE GENUS CREPIS

II. THE CHROMOSOMES AND TAXONOMIC
RELATIONSHIPS

BY

ERNEST BROWN BABCOCK
AND
MARGARET MANN LESLEY

UNIVERSITY OF CALIFORNIA PRESS
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CHROMOSOME NUMBER AND INDIVIDUALITY
IN THE GENUS *CREPIS*

II. THE CHROMOSOMES AND TAXONOMIC RELATIONSHIPS

BY

ERNEST BROWN BABCOCK AND MARGARET MANN LESLEY

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INTRODUCTION

For the past three years we have been accumulating data on the taxonomy and cytology of the genus *Crepis*. The present paper represents only two phases of our general project, which also includes extensive genetic research on species and species hybrids, the whole undertaking being an effort to establish a natural classification of a genus which has been a source of considerable difficulty to taxonomists and which presents a wide array of chromosome numbers. In addition to number we have examined the size of the chromosomes in the species studied, in the hope that this might also prove useful as a criterion in classification.

We are confining our discussion to species which we have been able to cultivate in the greenhouse or garden and to identify with certainty, a procedure which has thrown considerable light on the classification. Ideally the taxonomist should know his species as they appear under natural conditions, but obviously this is impossible for any one botanist in the case of such a large and widely distributed genus as *Crepis*.

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But, even though field studies of most of the species could not be made, it was yet necessary to cultivate them in order to study them cytologically, and hence it has been possible to supplement the examination of herbarium material by observations on cultivated plants which were grown under fairly uniform conditions. By this method it has been possible to show that certain characters (for example, nodding position of the young flower heads) which have been used by some authors to separate sections of the genus, are variable within a single species.

Crepis was chosen in the first place because certain species have small chromosome numbers and because the chromosomes are comparatively easy to study in some detail. A previous paper on chromosome size and number in the genus (Mann, 1925) contained a majority of the chromosome data herein considered, together with a suggestion as to how a cytologist would be tempted to group the species studied. In this paper we have added somewhat to the cytological data and have attempted to utilize both the cytological and the taxonomical modes of attack. Generally speaking, this method has proved of the greatest usefulness; and, while certain irreconcilable situations still appear to exist, we have reason to hope that future developments—as we obtain more species and make further studies—may show how such situations have arisen and lead the way to a clearer understanding of the genus.

MATERIAL AND METHODS

The species of *Crepis* upon which this study is based are all from the Old World, and have mostly been obtained through the coöperation of European botanists. Since we desire to make our study as complete as possible, we shall greatly appreciate any assistance towards obtaining viable seeds or roots of additional species. The taxonomic studies have included the examination of both dried and living specimens, and much care has been exercised in the determination of all this material. The cytological methods were described in Mann (1925).

ACKNOWLEDGMENTS

The investigations herein reported were conducted in part through an allotment from the Adams Fund. It is with pleasure that we acknowledge the assistance of Dr. J. L. Collins and Mr. C. W. Haney in the growing of cultures and in providing us with certain data on species hybridization. All the drawings were made by Helen E. Rearwin, whose attention to accuracy of detail is gladly acknowledged. Our thanks are also due to the curators of herbaria and directors of

botanic gardens in numerous institutions. Many taxonomic and other treatises on the Compositae have been consulted, which cannot be cited in this brief paper.

TAXONOMY AND CYTOLOGY OF TWENTY-ONE SPECIES OF CREPIS

In the present paper we do not wish to discuss the taxonomy of *Crepis* in detail or to propose any taxonomic revision of the genus, but merely to set forth the general features of the group and its subdivisions in such a way as to enable the reader to appreciate some of the difficulties involved in attempting to classify the species according to a natural system. Also, it is hoped that the significance of the cytological data herein presented will be clearer after a preliminary consideration of the outstanding morphological resemblances and differences to be found within this group of plants.

No thoroughgoing investigation of the entire genus has been made. Some of the species have been studied since the time of Linnaeus or even earlier, and at least forty-four other generic names have been applied by twenty-four authors in attempting to classify various portions of the assemblage. The purposes of the present paper can be best served by a discussion of the treatment of the genus given by Hoffmann in Engler and Prantl's *Pflanzenfamilien*. This treatment, represented in condensed form below, includes all but six of the twenty-one species for which complete data as to chromosome size are available and one other (*C. patula*) which we have not yet been able to secure. The six species referred to—*blattarioides* Vill., *bursifolia* L., *neglecta* L., *parriflora* Desf., *montana* d'Urville, and *setosa* Hall. f.—are all easily placed in Hoffmann's categories with the exception of *neglecta*, which is referred to **Eucrepis** in most recent floras (see p. 327). A translation of Hoffmann's description of the genus is given below for the information of readers who are not familiar with this group of plants. His analysis of the genus and key to the sections appear in table 1.

Crepis L.—Heads small to rather large, yellow- or seldom red-flowered, borne singly or in panicles of variable form; involucre cylindrical or bell-shaped, often with loose or appressed outer calyx, the inner fructiferous bracts often becoming stouter and harder throughout or along the middle nerve; receptacle naked or ciliate; fruit 10–30 ribbed, with a short callosity on the base, reduced or beaked at the apex, the outer fruits sometimes shaped differently from the inner ones; pappus in most species composed of soft pliable hairs, seldom somewhat brittle and brownish, in the marginal fruits sometimes lacking.—Herbs, very seldom half-shrubby plants. Perhaps 170 species mostly from the northern hemisphere.

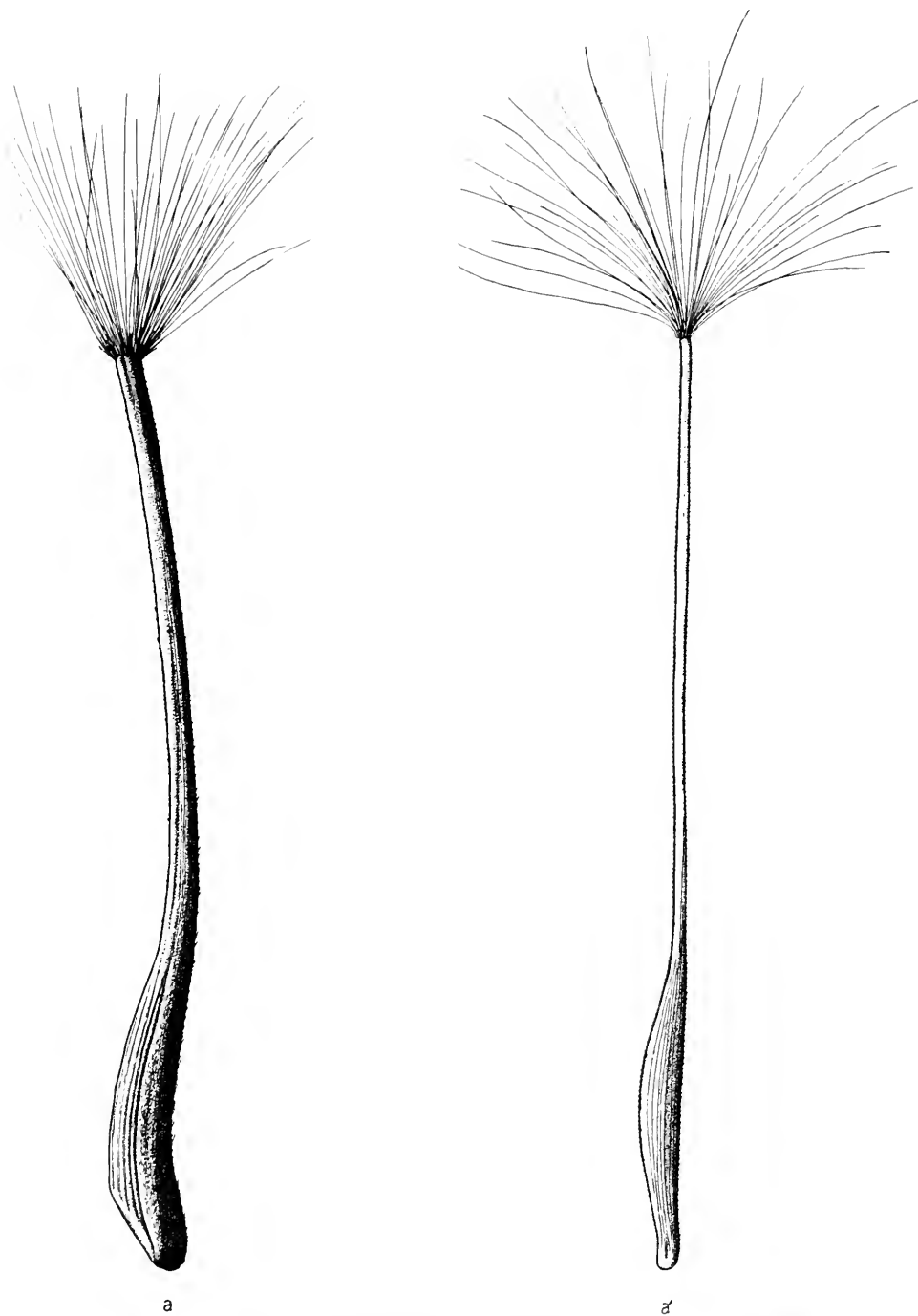


Fig. 1. Achenes of *Crepis alpina*—*a*, marginal; *a'*, inner. $\times 7$ circa.

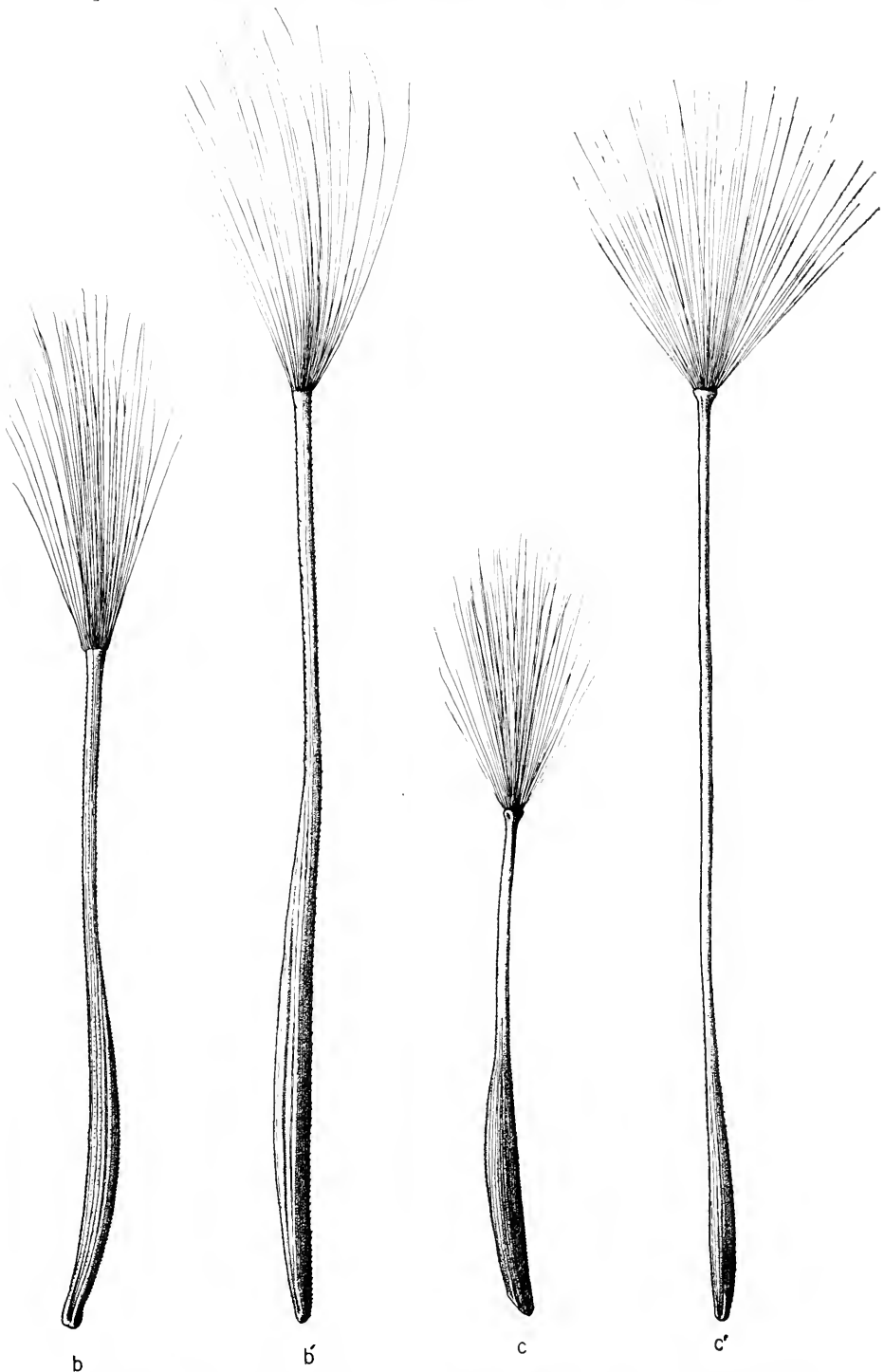


Fig. 2. Marginal and inner achenes of: *b, b'*, *Crepis rubra*; *c, c'*, *C. foetida*.
 $\times 7$ circa.

TABLE 1

HOFFMANN'S KEY TO THE SECTIONS OF *Crepis* WITH THE ADDITION OF SIX SPECIES NOT LISTED BY HIM AND REFERENCES TO ORIGINAL DRAWINGS OF ACHENES

A. Pappus bristles very short, unequal, the longest scarcely as long as the width of the fruit, very readily deciduous; fruit short-beaked.

Sec. I. **Ceramioccephalum** Schultz Bip.*

C. patula Poir.

B. Pappus bristles longer.

(a) Inner or all the fruits long-beaked.

Sec. II. **Barkhausia** Muhl.*

Fruits all beaked (outer sometimes shorter than inner), involucre mostly with outer calyx, seldom imbricate. Fig. 1, *a, a'*; Fig. 3, *d, e, e' g, g'*.

C. alpina L., *taraxacifolia* Thuill., *bursifolia* L., *setosa* Hall. f.

Sec. III. **Anisoderis** Cass.*

Outer fruits short-, inner long-beaked. Fig. 2, *b, b', c, c'*.

C. foetida L., *rubra* L.

Sec. IV. **Nemauchenes** Cass* (in part).

Marginal fruits not or scarcely beaked, enclosed within the much hardened involueral bracts; ribs prominent, the innermost enlarged wing-like so the fruits seem to be compressed; inner fruits prismatic long-beaked. Fig. 3, *h, h'*.

C. aspera L.

(b) Fruits reduced at the apex, but not beaked or only short-beaked.

Sec. V. **Nemauchenes** Cass.* (in part).

Except for the scarcely beaked inner fruits, like IV. Fig. 4, *k, k'*.

C. Dioscoridis L.

Sec. VI. **Cymboseris** Boiss.*

Marginal fruits compressed, 3-angled, the edges winged, enclosed by the inner much hardened involueral bracts, without pappus. Fig. 4, *m, m', m''*.

C. palaestina Boiss. (Bornm.).

Sec. VII. **Phaegasium** Cass.*

Fruits alike in shape with readily deciduous pappus which is mostly absent in the marginal fruits, inner fructiferous involueral bracts much hardened. Fig. 4, *n, n', n''*.

C. pulchra L.

Sec. VIII. **Aetheorrhiza** Cass.*

Distinct from others by tuberous root-stock, fruits all similar in shape. Fig. 6, *u*.

C. bulbosa (L) Tausch.

Sec. IX. **Eucrepis** DC.

Roots not tuberous (fusiform or root-stock as though bitten off); fruits all alike; involucre with outer calyx; inner fructiferous involueral bracts mostly moderately thickened. Fig. 5, *o, p, q, r, s, t*.

C. capillaris (L) Wallr., *neglecta* L., *parriflora* Desf., *tectorum* L., *biennis* L., *montana* d'Urv.

* Described as a genus.

Sec. X. **Youngia** Cass.*

Distinct from preceding section in the small few-flowered (8-15) heads. Stem few-leaved; involucre in mature fertile heads little changed. Pappus readily deciduous. Fig. 6, *v*, *v'*.

C. japonica (L) Benth.

Sec. XI. **Catonia** Mueh.*

Involucre imbricate, often black hairy; outer bracts shorter but at least half as long as inner bracts and forming no distinct outer calyx, in mature fertile heads flat and unchanged. Fig. 6, *w*, *x*; fig. 7, *y*.

C. sibirica L., *aurea* (L) Cass., *blattarioides* Vill.

We shall first discuss Hoffmann's grouping of the twenty-one species now before us, and then suggest a more natural grouping, in order that the cytologic data to be presented may be more intelligently considered. It will be noted that the genus, as treated by Hoffmann, is divided into three subgenera but without designating them as such. The first consists of the monotypic section, **Ceramiocephalum**; the second (*a*) contains three sections all characterized by having fruits with definite beaks; and the third (*b*), comprising the remaining seven sections, contains species none of which have manifestly beaked fruits. It was long ago pointed out (Bischoff, 1851) that all degrees of development of the beak are found in group (*a*), while some of the species included in group (*b*) have fruits with very short or obscurely developed beaks. But this seems to be generally looked upon as merely part of the evidence of relationship within the whole group and as part of the argument for treating it as a single genus.

Section I is set apart from all the other species, probably justifiably, but as we have not yet been able to work with living material of this interesting species, it is unnecessary to give it further consideration at present.

Subgenus (*a*), on the basis of fruit characters alone, would be better rearranged as follows:

Sec. II. Fruits large, the inner ones 10-18 mm. long.

C. alpina, *foetida rubra* (cf. figs. 1 and 2).

Sec. III. Fruits small, all alike, the inner ones 5-8 mm. long.

C. bursifolia, *setosa*, *taraxacifolia* (cf. fig. 3, *d*, *e*, *g*).

Sec. IV. Fruits small, of two shapes, marginal ones winged.

C. aspera (cf. fig. 3, *h*, *h'*).

Furthermore, the above rearrangement is not inconsistent with other morphological characters of diagnostic value. This is especially interesting in connection with the cytological evidence, the species

* Described as a genus.

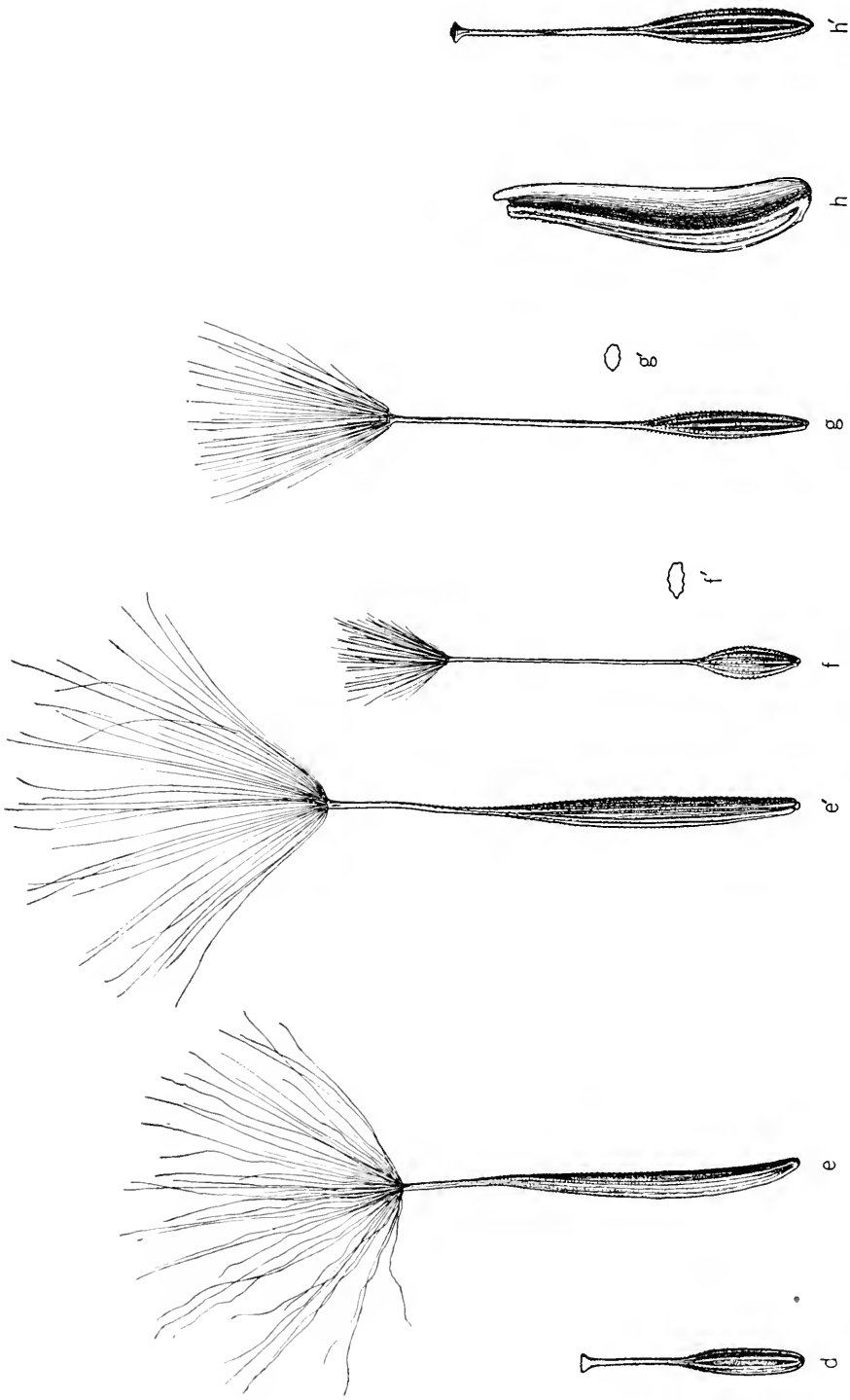


Fig. 3. *d*, typical achene of *Crepis setosa*; *e*, *e'*, marginal and inner achenes of *C. taraxacifolia*; *f*, typical achene of *C. senecioides*, *f'*, outline of cross-section of same; *g*, typical achene of *C. hirsifolia*; *g'*, outline of cross-section of same; *h*, *h'* marginal and inner achenes of *C. aspera*. X 7.5 *circum*.

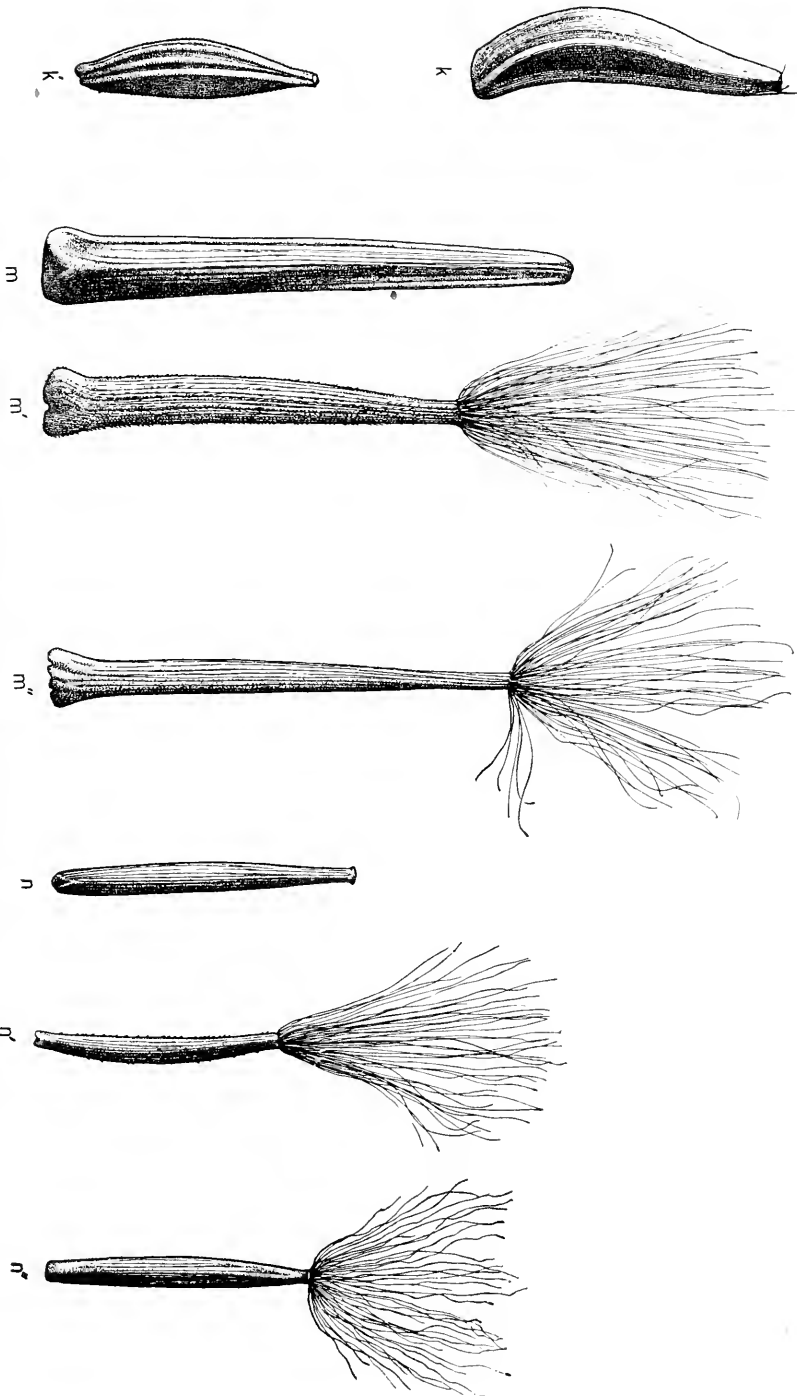


FIG. 4. *k*, *k'*, marginal and inner achenes of *Tropis Diascoridis*; *m*, marginal and *m'*, *m''*, inner achenes of *C. pulchra*; *n*, marginal and *n'*, *n''*, inner achenes of *C. pulchra* forma. X 7,000.

grouped under Section II all having 5 pairs of chromosomes of similar size, while those under Sections III and IV have 4 pairs but differ somewhat in individuality. It is worthy of note that one character commonly used in distinguishing between these species, viz., the position assumed by the young flower heads before anthesis, whether erect or nodding, has been found to be too variable in the case of *foetida* to make it of diagnostic value.

In its dimorphous fruits, the inner ones beaked and the outer ones winged, *C. aspera* exhibits relationship with **Barkhausia** on one side and the *Dioscoridis* group on the other (cf. fig. 4, *k*, *k'*). Its chromosome group resembles those of the three **Barkhausia** species in having chromosomes of medium size, and it has been crossed with two of these species. But these hybrids exhibit very abnormal reduction phenomena, whereas hybrids between certain **Barkhausia** species (*vesicaria*, *Marschallii* and *taraxacifolia*) show normal pairing and reduction. Thus all the evidence indicates that *aspera* belongs in a class by itself. Furthermore, *amplexifolia*, which closely resembles *aspera* morphologically, also has 4 pairs of medium-sized chromosomes (p. 331).

Subgenus (*b*) is a heterogeneous group which is scarcely capable of satisfactory classification on the basis of fruit characters alone. Thus in the case of sections V, VI, and VII there is much stronger affinity, as indicated by comparative morphology, than would appear from Hoffmann's synopsis. In all three of the species concerned the inner involueral bracts of fructiferous heads are conspicuously thickened or much hardened. Then, too, *palaestina* has a combination of some of the distinguishing characters of the other two species, and yet it is in no sense an intermediate form such as might arise from hybridization. The flower heads in *palaestina* are large and showy, and the marginal fruits are enclosed within the inner involueral bracts, in these respects resembling *Dioscoridis*, while the inner fruits bear a strong resemblance to those of *pulchra*. Furthermore, the fruits in *pulchra*, contrary to Hoffmann, are sometimes of two distinct shapes, the marginal ones being flattened as in *palaestina* (cf. fig. 4). Without going into further details at this time, we may suggest that these three sections might well be combined into one. The chromosome groups of *pulchra* ($N=4$), *palaestina* ($N=4$), and *Dioscoridis* ($N=4$) are indistinguishable, and the F_1 of *pulchra* \times *palaestina* is highly fertile.

Section VIII, **Aetheorrhiza**, must stand alone, at least for the present. While the inflorescence of *bulbosa* suggests strong relation-

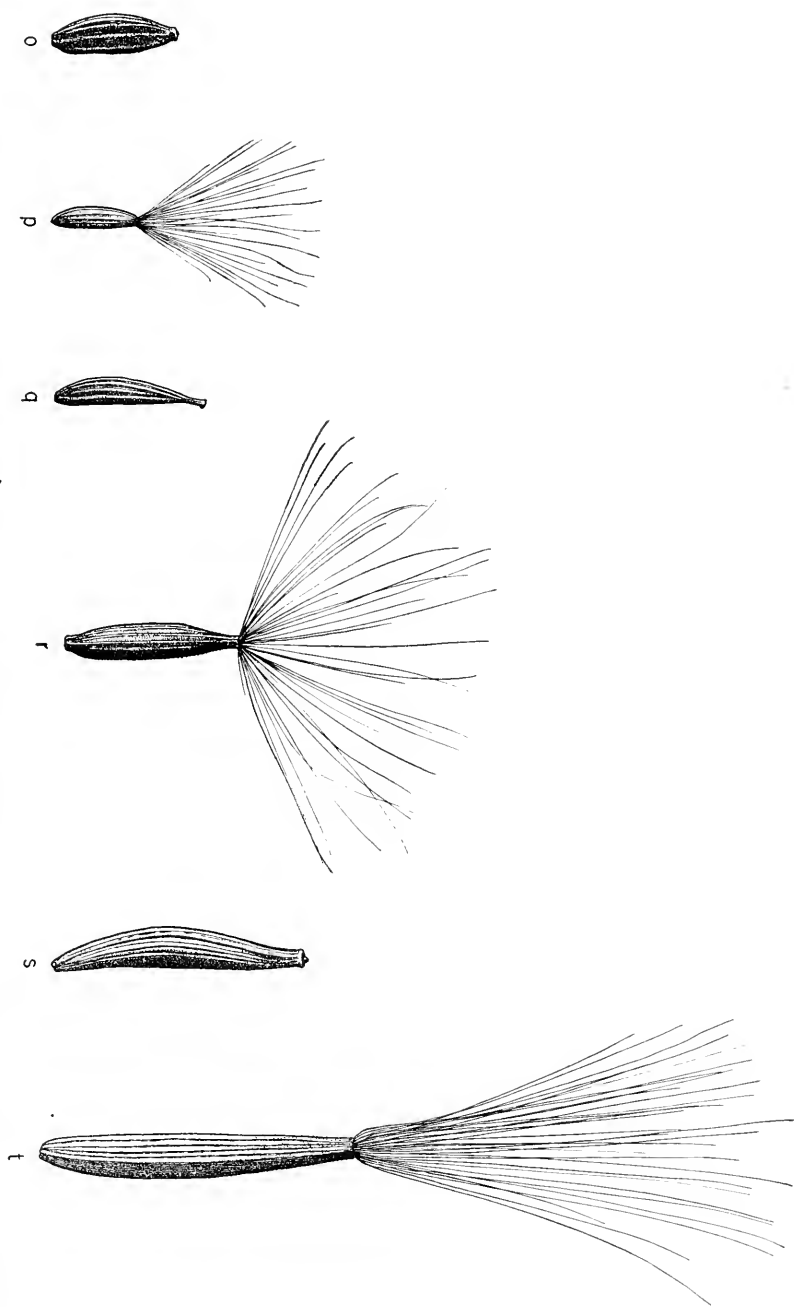


Fig. 5. Typical achenes of: o, *Crepis capillaris*; p, *C. parviflora*; q, *C. neglecta*; r, *C. tectorum*; s, *C. montana*; t, *C. bicornis*.
X 7. circa.

ship with *aurca*, this species is cytologically very different from all other species of *Crepis*, having 9 pairs of short chromosomes. The only species studied which it at all resembles in this respect is *japonica*, which has 8 pairs of chromosomes of similar size.

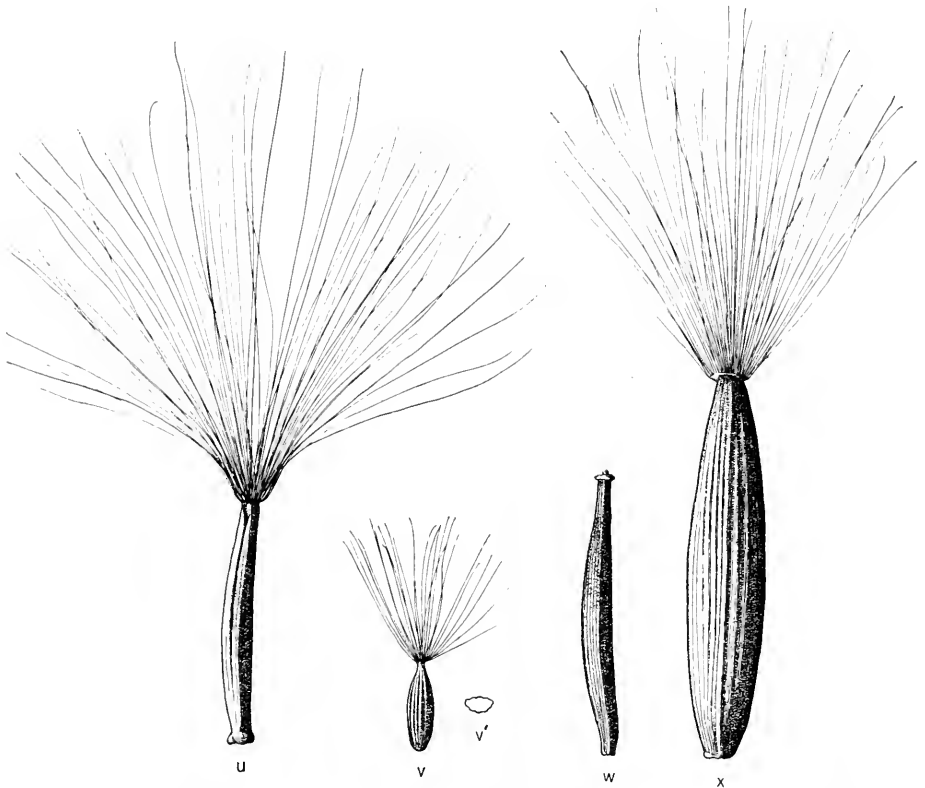


Fig. 6. Typical achenes of: *u*, *Crepis bulbosa*; *v*, *C. japonica*—*v'*, cross-section outline; *w*, *C. aurca*; *x*, *C. blattarioides*. $\times 6.5$ circa.

Section IX. **Eucrepis**, contains six of our twenty-one species, and on the basis of fruit characters alone (cf. fig. 5) they comprise three groups, as follows: 1. *capillaris* and *parviflora*; 2. *neglecta*, *tectorum*, *montana*; 3. *biennis*. But if we consider habitual and other morphological characters, they may be rearranged as follows: 1. *capillaris*, *parviflora*, *neglecta*; 2. *tectorum*; 3. *biennis*; 4. *montana*. Such an arrangement is of interest when considered in relation to the chromosomes of these species. It was noted (Mann, 1925) that the total length of the chromosome group in *capillaris* ($N=3$) is practically the same as that of *neglecta* ($N=4$), while *parviflora* ($N=4$) appears to have a short chromosome added to a complex like that of *capillaris*. The chromosome group of *tectorum* ($N=4$) could not be differentiated

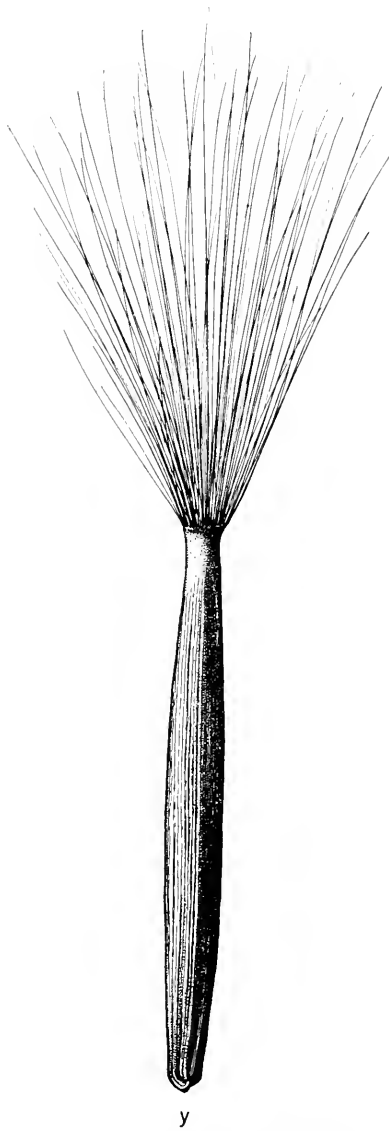


Fig. 7. Typical achene of: *y. Crepis sibirica*. $\times 7$ circa.

from that of *taraxacifolia* in **Barkhausia**, but *biennis* ($N = 20$) and *montana* ($N = 6$) stand apart from all other species from the standpoint of chromosome number.

It should be observed that *C. neglecta* has long been a troublesome species to students of this difficult genus. In the *Genera Plantarum* (Bentham and Hooker, 1873) *neglecta* is considered as intermediate

between **Eucrepis** and **Lagoseris (Barkhausia)**; *parviflora* was given similar intermediate status, but this is manifestly an error. In the *Flora Orientalis* (Boissier, 1875) we find a statement which we translate as follows: "As the achenes gradually diminish into a short beak, it is doubtful whether this species belongs in **Eucrepis** or **Barkhausia**; it affords a connecting link between the two sections." Boissier places it under **Barkhausia**, presumably because the young flower heads assume a nodding position. The unreliability of this character has been pointed out. Moreover, recent taxonomists (e.g., Fiori, 1904) have placed *neglecta* in **Eucrepis**, where it seems to belong rather than in **Barkhausia**, as its fruits are variable in shape and even when they are beaked the beak is very short, as shown in figure 5*q*.

Section X, **Youngia**, is represented here by only one species, but contains several others, of which one is *fuscipappa* (p. 331). These comprise a very distinct group in certain morphological characters, inasmuch that some authors have suggested placing it in *Lactuca*. But it is claimed (Bentham and Hooker, 1873; Hooker, 1882) that the species of this group (except two referred to *Lactuca* or *Iceris*) resemble **Eucrepis** more closely than *Lactuca*, and that *japonica*, which is the type species of Cassini's genus, *Youngia*, does not differ much in floral characters from *C. parviflora*, a statement which is partially true, although a number of differences do exist. It was noted above that *japonica* ($N=8$) resembles *bulbosa* in having very short chromosomes. It is the only species known in the genus with 8 small chromosomes (*japonica* chromosomes total about 93 units in length as compared with 137 for *fuscipappa*) and it was shown in Mann (1925) that considering chromosome size alone it might have been derived from *tectorum* (**Eucrepis**) by cross-division of all chromosomes. However, these two species are so widely different morphologically that such a derivation seems hardly possible. On account of the strongly flattened fruits in *japonica* (cf. fig. 6, *v*, *v'*), together with the other differences noted in Hoffmann's key and the small size of the chromosomes, one may advocate the recognition of Cassini's *Youngia* as a genus intermediate between *Crepis* and *Lactuca*. Cassini (1831) in the original diagnosis of *Youngia* states: "fruits oblong, more or less flattened, . . . absolutely beakless" . . . [genus] "not to be confounded with *Crepis* because of the flattened fruits." Further comparative study of shape of fruits and size of chromosomes will be necessary, however, before a final conclusion can be drawn.

TABLE 2

TENTATIVE CLASSIFICATION OF TWENTY-ONE SPECIES OF *Crepis*, ARRANGED FOR COMPARISON WITH HOFFMANN'S CLASSIFICATION SHOWN IN TABLE 1

B. Pappus bristles longer.

1. Inner or all the fruits long-beaked.

2. Fruits large, the inner ones 10-18 mm. long.

Sec. II. **Anisoderis.**

C. alpina, foetida, rubra (figs. 1 and 2).

2*. Fruits small, the inner ones 5-7 mm. long.

3. Fruits all similar.

Sec. III. **Barkhausia.**

C. bursifolia, setosa, taraxacifolia (fig. 3, *d, e, g*).

3*. Fruits of two shapes, the marginal ones winged.

Sec. IV. **Nemauchenus.**

C. aspera (fig. 3, *h, h'*).

1*. Fruits reduced at apex, but not beaked or only short-beaked.

4. Inner involueral bracts conspicuously thickened or hardened in fructiferous heads.

Sec. V. (**Gatyona, Cymboseris, Phaecasium.**)

C. Dioscoridis, palaeestina, pulchra (fig. 4).

4*. Inner involueral bracts not much thickened or hardened in fructiferous heads.

5. Inner involueral bracts more or less spongy-thickened dorsally.

Sec. VI. **Eucrepis.**

C. capillaris, parviflora neglecta, tectorum, biennis, montana (fig. 5).

5*. Inner involueral bracts little or not at all changed.

6. Heads small, florets few, small.

Sec. VII. **Youngia.**

C. japonica (fig. 6, *v, v'*).

6*. Heads large, florets numerous, large.

7. Plant short-stemmed, scapigerous, scapes 1-headed, rarely 2-3 headed.

8. Rootstock stoloniferous, forming tubers.

Sec. VIII. **Aetheorrhiza.**

C. bulbosa (fig. 6, *u*.)

8*. Rootstock simple, non-tuberous.

Sec. IX. **Omalocline.**

C. aurea (fig. 6, *w*).

7*. Plant long-stemmed, erect, foliate.

Sec. X. **Soyeria.**

C. sibirica, blattarioides (fig. 6, *x*; fig. 7, *y*).

Section XI. **Catonia**, is defined by Hoffman as including species of at least two distinct groups, **Omalocline** Cass. and **Soyeria** Mann., represented among our species by *aurea* on the one hand and by *blattarioides* and *sibirica* on the other. In other words, he has used an ill-defined genus (Moench, 1794) as a catchall for species not already assigned to sections. This would be more evident if we were considering a larger number of species. Furthermore, *blattarioides*

and *sibirica*, although somewhat similar in both habit and fruit characters (see figs. 6, 7), are very distinct from each other in many respects and have the same general native and distributional habitats, all of which would indicate that they are not closely related species. The three species of **Catonia** studied differ greatly cytologically. *Aurea* ($N=5$) is rather different in individuality from the other species with 5 pairs. *Blattarioides* ($N=4$) has a chromosome group much like that of *tectorum*, while *sibirica* has 5 pairs of very large chromosomes resembling those of *Dioscoridis*, *pulchra*, and *palaestina*. Three other species in this section have been counted recently, but as no measurements have yet been made, they are not included in table 3 (see p. 331).

TABLE 3

TABULATION OF TWENTY-ONE SPECIES OF *Crepis* ACCORDING TO A TENTATIVE NEW TAXONOMIC GROUPING AND WITH REFERENCE TO NUMBER AND LENGTH OF CHROMOSOMES. (THE LENGTH VALUES REPRESENT AVERAGES FROM TEN DIFFERENT CELLS.)

	Number of Chromosome Pairs								
	1	2	3	4	5	6	7	8	9
Sec. II. Anisoderis									
<i>alpina</i>	26.2	21.3	14.5	13.1	12.2				
<i>foetida</i>	25.0	20.8	17.7	15.8	14.4				
<i>rubra</i>	29.4	23.9	18.5	16.2	14.9				
Sec. III. Barkhausia									
<i>bursifolia</i>	24.3	22.0	19.5	12.7					
<i>setosa</i>	22.3	17.8	14.0	9.1					
<i>tarazacifolia</i>	26.1	23.3	21.2	17.8					
Sec. IV. Nemauchenes									
<i>aspera</i>	23.9	21.5	19.7	17.5					
Sec. V.*									
<i>Dioscoridis</i>	35.9	29.3	24.9	19.3					
<i>palaestina</i>	34.1	27.0	24.6	21.2					
<i>pulchra</i>	36.7	30.6	25.5	19.3					
Sec. VI. Eucrepis									
<i>capillaris</i>	26.2	20.4	14.8						
<i>parviflora</i>	25.3	20.5	14.4	9.7					
<i>tectorum</i>	28.1	23.2	20.2	17.2					
<i>montana</i>	26.8	21.4	17.7	16.0	15.2	12.5			
<i>biennis</i>	(20 pairs)†								
Sec. VII. Youngia									
<i>japonica</i>	15.7	13.5	12.2	11.5	10.8	10.0	9.7	9.2	
Sec. VIII. Aetheorrhiza									
<i>bulbosa</i>	13.9	12.8	12.1	11.7	11.1	10.6	10.1	9.6	8.6
Sec. IX. Omalochine									
<i>aurea</i>	21.0	18.0	16.2	15.1	13.2				
Sec. X. Soyeria									
<i>sibirica</i>	41.9	32.4	27.6	23.2	18.5				
<i>blattarioides</i>	29.0	23.8	20.6	17.7					

* *Gatyna*, *Cymboseris*, and *Phaeacasium* combined.

† Not measured; size range much like that of species in this group.

Our analysis of relationships among these twenty-one species, as based on comparative morphology, is summarized in table 2. This analysis is presented only in a tentative way, as an aid in the study of cytological evidence and a step toward the classification of the entire genus.

The correspondence of the new taxonomic grouping with chromosome number and size is shown in table 3.

Since the foregoing was written, the chromosomes have been examined in the following additional species of *Crepis*. The classification into sections is according to the tentative new arrangement shown in tables 2 and 3.

IV. *Nemauchenes*

C. amplexifolia (Godr.) Willk. N = 4 size medium

VI. *Eucrepis*

{ <i>C. lyrata</i> Froel.....	N = 6	size medium
{ <i>C. mollis</i> (Jacq.) Asch.....	N = 6	size medium
<i>C. pygmaea</i> L.	N = 6	size medium
{ <i>C. chondrilloides</i> Jacq.	N = 4	size large
{ <i>C. Blavii</i> Asch.	N = 4	size large
<i>C. ciliata</i> C. Koch.....	N = 20	size medium

VII. *Youngia*

C. fuscipappa (Thw.) Benth..... N = 8 size medium

IX. *Omaloclone*

C. Hookeriana Ball..... N = 4 size medium

X. *Soyeria*

<i>C. conyzaefolia</i> (Gouan) Dalla Torre.....	N = 4	size large
<i>C. tingitana</i> Salz. ex Ball.....	N = 5	size medium
<i>C. paludosa</i> (L) Mneh.....	N = 6	size large

With reference to the six species classified under *Eucrepis*, the first group of three *lyrata*, *mollis*, and *pygmaea*, must be grouped with *montana* on the basis of morphology, and they have similar chromosomes. The next two, *chondrilloides* and *Blavii*, represent a subdivision of *Eucrepis* not previously studied and are very distinct from other members of *Eucrepis*. Lastly *ciliata* is certainly in *Eucrepis*, and its chromosomes indicate relationship to *biennis*, to which species there is considerable resemblance in the rosettes of our immature plants. Evidently *Eucrepis* is too heterogeneous a group to be retained as a section, and in the taxonomic revision of the genus which is now in preparation it will become a subgenus containing several sections.

It is evident that, generally speaking, there is a definite correspondence between the taxonomic position of the species studied and their chromosome number and especially with chromosome size, and that the new taxonomic grouping increases this correspondence. It is almost perfect in Section II, and in Section III (cf. table 3), and the species that stand apart in the classification also differ markedly from the rest in either size or number of chromosomes (Sections V, VI, and VII). It will be noted that Section III and Section VI contain species with similar chromosome numbers and sizes, *parviflora* and *setosa* having very similar size differences, as do also *taraxacifolia* and *tectorum*. It would seem worth while to test these groups by means of species-hybridization. Sections VII and VIII as compared with Sections V and X exhibit the most extreme differences in chromosome size.

LITERATURE AND DISCUSSION

The numerous summaries of chromosome numbers which have appeared in recent years clearly indicate that there is some parallelism between chromosome number, size, and shape and relationship in the plant and animal kingdoms. In general, members of the same genus usually have similar chromosome numbers. In the Liliaceae, for instance, each genus has a characteristic number of chromosomes. On the other hand, in wheat, instead of exact numerical correspondence within the genus, the species fall into three groups with respect to chromosome number (Sakamura, 1918), einkorn having 7, emmer 14, and vulgare 21 pairs of chromosomes. These groups also differ from one another in susceptibility to rust, serological relations, and morphology (Sax, 1921). Thus in the genus *Triticum* the most similar species are most alike in chromosome number. Winge (1917, pp. 166-168) cites an interesting case from the Compositae. Species were described as having 8, 9, 14, 16, 18, 24, 27, 32, 36, and 45 pairs. When these species were classified by tribes, the numbers formed two series with 8 as the ground number for the Heliantheae, and 9 for the Anthemideae. Marchal (1920) recently noted that the species of the genus *Campanula* which belong to the section Medium have N values of 17, 34, or 51, but finds that the other section of the genus fails to show a similar numerical seriation, including such N values as 8, 10, and 13. He suggests (p. 66) that "The results of the cytological study of species of section II [Rapunculus] tend to show that this grouping is much less natural and less homogeneous than the preceding."

McClung (1908), on the basis of observations on many genera of Orthoptera, says,

Merely as a result of the study I have made of the germ cells I would have classified these insects into two groups, one having a complex of twenty-three chromosomes and the other of thirty-three. On the other hand, many taxonomists, from careful and minute examination of the external anatomy of these same species, had agreed in placing them into family groups which they call the Acrididae and Locustidae.

McClung (1917) has made an especially thorough study of the genera *Hesperotettix* and *Mermiria*, and has had the benefit of the coöperation of experts on the classification of the Orthoptera, with similar results.

Metz (1914, 1916) has shown that the Drosophilidae have rather similar chromosomes and that the species form several groups on the basis of their cytological characteristics. Metz and Lancefield (1922) state that the 13 species belonging to class A, of which *D. melanogaster* is an example, are scattered throughout the genus. The Drosophilidae are of especial interest from the standpoint of cytology and taxonomy, since something is known of the arrangement of genes within the chromosomes of several species, and it is therefore possible to compare the chromosomes from a genetical as well as a purely morphological viewpoint. Sturtevant (1921) says, "44 recessive mutant genes in 41 loci of *D. melanogaster* and 12 recessive mutant genes of *D. simulans* (in 12 loci) are also recessive in *melanogaster-simulans* hybrids." Some of these genes are found in each of the 4 chromosomes indicating that "The data from *D. simulans* show what was suggested by the other results and by much cytological data, that the constitution of a chromosome may be essentially the same in two different species." Both of these species belong to type A *cytologically* (Metz and Moses, 1923) and are closely related taxonomically. The evidence from *D. obscura* and *D. willistoni*, on the other hand, shows that the chromosomes which one would naturally suppose to be identical on the basis of purely cytological criteria are not the same genetically, since Metz and Lancefield (1922) state: "In the two species having V-shaped X chromosomes, then, yellow and seute are 'located' near the middle of the chromosome map, while in *melanogaster* with its short rod-like X chromosome, yellow and seute are on one end." Metz and Moses (1923) emphasize the importance of genetical evidence in any attempt to evaluate the significance of similarities or differences of a cytological type.

Lists of chromosome numbers also contain what appear to be many flagrant exceptions to the view that the species of a genus will be cyto-

logically similar. In fact, the summaries of Ishikawa (1916) and Tischler (1916, 1922) contain very few genera with either the same number throughout, or even a single ground number. Even in the Liliaceae certain species have been reported as having chromosome numbers different from that typical of the genus. Time and further work alone will tell how many of these exceptions are real and how many are due to error. At present few genera have been much studied, and even where a large number of counts have been published, the same error may appear in a whole series of observations. For instance, in both *Triticum* and *Rosa* numerous species were included in recent summaries as having 8 and 16 pairs of chromosomes. It has been shown by Sakamura (1918) and Sax (1918, 1921) for *Triticum*, and by Täckholm (1922) for *Rosa*, that 7 and not 8 is the ground number for both genera. Another very real source of error in any attempt to generalize from summaries lies in the fact that few cytologists are trained taxonomists. Our experience with *Crepis* indicates that seeds which are obtained from the most reputable sources may be incorrectly labeled, and, unless the seeds are grown and the plants classified, we cannot always be positive that they even belong to that genus, much less to the species to which the sender has attributed them. While lists of chromosome numbers include such errors as are indicated above and are, therefore, not suitable as a basis for very sweeping generalization, no one can doubt that chromosome number and, in some cases, size and shape, are good specific characters. We venture the prediction that chromosome number and size will sometime be given with taxonomic descriptions.

Crepis contains species with 3, 4, 5, 6, 8, 9, and 20 pairs of chromosomes; but 3, 6, 8, 9, and 20 are much less frequent numbers than 4 or 5, each of the former characterizing only one of the twenty-one species represented in table 3. A similar condition has been described for a closely related genus, *Lactuca* (Ishikawa, 1921), most of the species having 5, 8, 9, or 12 as the haploid number, while single species have 7, 16, or 24. It is especially interesting that Ishikawa finds that his grouping of species according to chromosome number and size corresponds very strikingly with the taxonomic classification of Nakai (1920). In *Lactuca*, as in *Crepis*, great differences in chromosome size exist, and because of this and the numerical differences, Ishikawa is inclined to think that *Lactuca* is really an assemblage of genera. It is particularly interesting that two varieties of *L. dentata* have 12 pairs, while one has 7 pairs of chromosomes.

Crepis senecioides Delile, a native of Egypt, is a species of peculiar interest because its fruit is definitely flattened, although not so much so as in the more extreme types of *Lactuca*, and it lacks the thin lateral margin (fig. 3, *f*, *f'*), while on the basis of its involucre, number of florets per head, and habit it does not fit into any of the sections of *Lactuca* provided by Hoffmann in the Pflanzenfamilien. Furthermore, it has four pairs of small chromosomes and produces sterile hybrids when crossed with *C. parviflora* and *C. vesicaria*. Thus we find fairly close relationship between what simulates *Lactuca* in achene shape and certain species of *Crepis*. This evidence is not unique, however, as there are other points at which the two genera meet. Nakai, for example, found it necessary to choose between the alternatives of either recognizing *Ixeris*, *Paraxeris*, and *Crepidiastrum* as distinct genera or combining *Crepis* and *Lactuca*. For the present, we are inclined to consider *C. senecioides* as *Crepis*, but it is highly desirable that critical comparison of the fruits be made between *senecioides* and similar *Crepis* species as well as between *senecioides* and the North African species of *Lactuca*, and that chromosome counts of the latter be obtained. We have indicated one such comparison in the drawing of *C. bursifolia* (fig. 3, *g*, *g'*).

A group of forms which have usually been treated as distinct species, viz., *Crepis vesicaria* L., *C. taraxacifolia* Thuill., *C. Marschallii* F. Schultz, and *C. myriocephala* Coss. et DR., may be considered as one species for the following reasons: (1) They are closely similar morphologically, and their close relationship has been recognized by several taxonomists. (2) They have nearly identical chromosome groups. (3) They intercross freely and produce highly fertile hybrids. That these should be considered as subspecies of one species rather than as varieties is indicated by the following facts: (1) All except one, *taraxacifolia*, which is probably the oldest phylogenetically, occupy distinct geographic areas. (2) All are highly variable, and *taraxacifolia* is really polymorphous. However, as no changes in nomenclature are proposed in the present paper, we shall continue to use the binomials in what follows.

A summary of the data recently presented by Bleier (1925) and Karpetchenko (1925) shows that in *Trifolium* section **Chronosemium***

* Greene (1897) discusses at length the evidence for retaining the genus *Chrysopsis* instead of treating it as a section (**Chronosemium**) of *Trifolium*. He says: "And since Linnaeus' time there have been a number of open protests, and by most able botanists, against the treating of the Hop Trefoils as congeneric with such plants as *Trifolium pratense* and its allies. Systematists of no less renown than Lamarek and Desfontaines referred the plants to *Melilotus* rather than *Trifolium*."

contains species with 7 or 14 pairs of chromosomes, while **Enamoria** and **Galearia** consist of species with 8 or 16 pairs, except for *T. glomeratum* which has 7 pairs; whereas **Lagopus** contains species with 7, 8 or a large number of pairs, possibly 48-49. Bleier presents some evidence that differences in nuclear volume and in chromosome size occur in the genus. The cases of *Trifolium*, *Campanula*, *Lactuca*, and *Crepis* are alike in that, while many correspondences have been found between chromosome number and classification, some exceptions still exist which require further study. Even within **Eucrepis**, however, which shows a remarkable diversity of chromosome numbers, morphological resemblances appear within the section which are correlated with similarity of chromosome number and size.

In the genus *Senecio*, Afzelius (1924) reports a high degree of homogeneity within the genus as indicated by close conformity to the numerical series, 5, 10, 20, 30; also in most of the sections, as only one of the eight sections contains species of different numerical rank. However, as the species he has studied are mostly from the Old World, the situation within the genus as a whole may yet be found to differ considerably.

In *Carex*, Heilborn (1924) has recently reported that species exist with 9, 15, 16, 19, 24, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 40, 41, 42, and 56 as haploid numbers. Related species show some numerical similarity, although this is by no means so striking as in *Lactuca*.

Crepis also contains a series of chromosome numbers like that reported for *Carex*, 3, 4, 5, 6, 8, 9, and 20 pairs. Most of the species with 3, 4, 5, 6, and 20 pairs have chromosomes similar in size, although some 4- and 5-paired species have chromosomes that are much larger than is usual in *Crepis*, in so far as it has been studied cytologically. Two of the three species which we have found with 8 and 9 pairs have much smaller chromosomes than is usual in the genus. It was noted above that the section **Youngia** might be removed from *Crepis*. If this is done we shall lack species with 8 pairs. It is noteworthy that **Eucrepis** contains species with 3, 4, 5, 6, and 20 pairs. Navashin (1925*b*) and Collins and Mann (1923) found evidence that polyploidy occurs in *Crepis*, but it was pointed out by Mann (1925) that some other type of chromosome multiplication must account for the origin of most of the species which we have studied. Non-disjunction was first suggested as a source of the chromosome differences observed by Rosenberg (1918); and, whereas this cannot account for all the differences, it may be the most important factor. In any case it certainly

is the most probable method which we know occurs. It should be emphasized in all such discussion, however, that there is *no known case of a stable combination of chromosomes which has been observed to originate in this way*. Similarly, no case of changed individuality of the chromosomes which would account for stable types like *C. setosa*, *neglecta*, and *parviflora* has been reported to have occurred experimentally. Chromosome fragmentation is known to occur following trisomy, but whether such types ever become stabilized with a pair of fragments added to the normal specific complex, or whether a chromosome complex can lose a considerable section of a pair of chromosomes and the plants lacking this part be viable and fertile, is unknown. Our strain of *C. Marschallii* is peculiar in that, when we obtained it, certain plants contained 9 chromosomes in the root-tip cells, comprising the usual complex for the *vesicaria* group of species plus a very short unpaired chromosome. The source of this small extra chromosome is quite uncertain, although it is known to be an addition to the complex. Navashin (1925) presented a figure of *C. Marschallii* that is like *vesicaria* and lacks the small chromosome. Some of our 9-chromosome *Marschallii* plants were very fertile, and among their progeny one at least has two such small chromosomes. This matter is being studied further and will be reported upon separately. Should such a plant be fertile, we might understand how such differences in chromosome groups could arise in a genus.

Navashin (1925a) has emphasized the importance of minute "Trabanten" or satellites attached to the tips of certain chromosome pairs in *Crepis* species. He believes that shape of chromosome and the presence or absence of satellites is "weit wichtiger für die Charakteristik des Kernes bzw. der Art, als die Zahl der Chromosomen und deren Dimensionen sind." He groups together in class "D" all chromosomes having satellites although in *C. Dioscoridis*, one of 19 length units bears the satellite, while in *C. parviflora* he finds it upon one of about 10 length units. But in our material, which was fixed in C. A. U., *Trabanten* were not always present, and sometimes resembled the strands and masses of nucleolar material which are frequently found being extruded from the chromosome plate. Consequently size, which is relatively far less variable and more easily evaluated, was selected as the best criterion of relationship, and it has thus far proved a very good one as tested by species-hybridization. That shape relationships may help in differentiating two pairs of chromosomes of the same size in certain species of *Crepis* is clearly indicated by Navashin's figures.

but the relative importance of size and shape as indicators of relationship between species can be tested only by species-hybridization and genetic analysis. Probably both modes of attack will sometime prove useful, but thus far they have not given us clues to relationship which could not be determined by comparative length alone. Our material, like that of Navashin, shows *Trabanten* attached to the shortest chromosome in both *tectorum* and *Marschallii*, species which are widely separated in all classifications. This is very disappointing, since one might have hoped that they could be differentiated thereby. It seems evident from our studies that if Navashin were to make comparative measurements of the chromosomes, he might change his estimate of the chromosome homologies in the species which he studied.

CORRECTIONS IN NOMENCLATURE IN PART I

In the preceding paper (Mann, 1925), the following corrections should be made:

For *brevisflora* Delile read *senecioides* Delile.

For *grandiflora* Tausch read *conyzaeifolia* (Gouan) Dalla Torre.

For *Sieberi* Boissier read *montana* d'Urville.

SUMMARY AND CONCLUSIONS

1. Taxonomically considered, the genus *Crepis*, as it stands at present, is a heterogeneous assemblage of distinct but related groups of species. The sections recognized by Hoffmann and their classification by him are not wholly satisfactory on the basis of comparative morphology alone. A more satisfactory classification of the species under consideration, which reduces the sections from eleven to ten and regroups certain species, is suggested, and the cytological evidence is considered in relation to the new grouping.

2. From the standpoint of cytology as well, the genus *Crepis* must be considered as heterogeneous. Similarity of chromosome size seems to be a better criterion of relationship than number alone, although closely related species usually have the same numbers of chromosomes. Most of the cytological heterogeneity is confined to the sections **Eucrepis** and **Catonia** of Hoffmann's classification. The former is found to be too heterogeneous both taxonomically and cytologically to be retained as a section, and certain new subgroupings are needed within it. **Catonia** also requires some drastic changes. It is hoped that further study will reveal natural subgroups within **Catonia**; also

that it may throw light on the origin of chromosomal differences in *Crepis*. Further research on species hybrids is in progress and should throw considerable light on problems of relationship within the genus.

3. Differences in chromosome dimensions are found among the species of this genus. We note especially (*a*) differences in size of all the chromosomes; (*b*) similarity in size of most of the chromosomes and differences in others. If **Youngia** be omitted, there remains only one species, *C. bulbosa*, having all the chromosomes smaller than is usual for the genus. At present we have this species in a section by itself, but its ultimate classification awaits further study. Of the three species of type (*b*), in which certain chromosomes are much shorter than is usual in the genus and the others are similar in size, *C. neglecta* and *C. parviflora* are provisionally classified in **Eucrepis**, while *C. setosa* is in **Barkhausia**.

4. It is noted that certain species having similar chromosome sizes, particularly *C. tectorum* and the *vesicaria* group (including *taraxacifolia*, *Marschallii*, and *myriocephala*), are classed respectively in **Eucrepis** and **Barkhausia**. These facts may indicate either close relationship between the two sections or that similar changes in the chromosomes have taken place independently in the two groups. For the present we favor the latter assumption.

5. This study was undertaken partly for the purpose of testing the cyto-taxonomic method in a genus favorable for such research. As the work progresses we are becoming more and more impressed with the value of this method, and it is our intention to extend it to include as many species of *Crepis* as can be obtained and cultivated at Berkeley.

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CHROMOSOMAL CHIMERAS IN CREPIS

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CHROMOSOMAL CHIMERAS IN CREPIS

BY

LILLIAN HOLLINGSHEAD

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Gates (1924) stated that "it is unknown at the present time how widespread polyploidy in somatic tissues may be." Several writers have since that time reported cases of somatic polyploidy and it was thought worth while to put on record instances of this condition recently discovered in the Genetics Laboratory of the University of California.

In the course of an examination of root tips of various *Crepis* species and species hybrids, two plants which were partly tetraploid have been found.¹ These are not the first cases of chromosomal chimeras reported in *Crepis*, Lesley (1925) and Nawaschin (1926) having previously recorded the phenomenon. Lesley's report is a note stating that in an F_1 between *C. biennis* ($n=20$) and *C. foetida* ($n=5$) a few neighboring cells were found having about twice 25 chromosomes, whereas most of the cells contained the expected 25. Nawaschin reported the occurrence of a tetraploid area in the form of a narrow sector in a diploid root of *C. Dioscoridis* ($n=4$).

The first of the two cases to be described was that of a chimeral root of a derivative from a cross between *Crepis biennis* ($n=20$) and *C. setosa* ($n=4$) which had 24 chromosomes in most of the somatic cells. In this root, however, a large number of cells was found which obviously had many more than the normal 24 chromosomes, several approximated 48, and two cells gave clear accounts of 48 chromosomes. The normal and tetraploid chromosome complexes are shown in figure A. By an examination of successive sections it was determined that the tetraploid cells were confined to a definite region which extended from at least very close to the root cap to a point where no division figures could be found. Apparently the longitudinal outlines of the tetraploid area were fairly regular, since only one case occurred in which diploid and tetraploid cells were found in different sections occupying the same position relative to the circumference, and this was on the line of demarcation between the two areas.

¹ Since writing the above a root of *C. Hakelei* ($n=8$) containing several neighboring cells with about twice the normal chromosome number and one of *C. montana* ($n=6$) with one tetraploid cell have been found.

Figure B was made up from an examination of the whole root and shows that the tetraploid area occupied the major portion of the root and that its cross-section was very irregular in shape. The dotted lines indicate the portions of the boundary between the $2n$ and $4n$ areas which could not be accurately determined. It is uncertain whether the tetraploid area extended into the central cylinder or not. The tetraploid area at a is two cell layers deep, at b it is only one, but opposite a at c it occupies most or all of the cortex. While there is considerable variation in cell size within both areas the average size of the tetraploid cells is larger than that of the diploid.

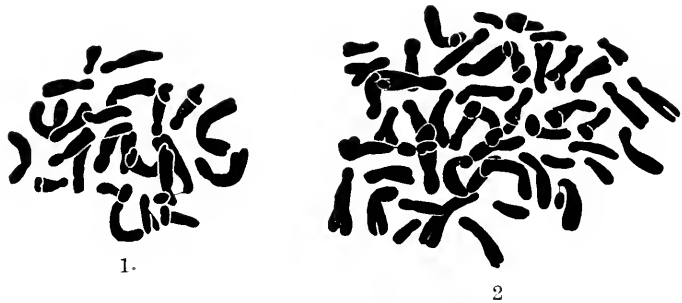


Fig. A. 1, Diploid chromosome complex of *biennis-setosa* hybrid derivative ($2n=24$). 2, Tetraploid complex ($2n=48$) from chimeral root of the same plant.

The shape of the tetraploid area is of some interest from the point of view of development as presumably the doubling of chromosomes took place in one of the initial cells from which the root developed. The area does not show the comparative regularity in shape exhibited by Nawaschin's tetraploid sector. On the other hand, tetraploidy here is confined to one definite area, which was not the case with Lesley's (1925) tomato chimeras, where isolated areas of tetraploid cells were observed. The condition is similar to that which Langlet (1927) found in two roots of *Thalictrum* but differs from that reported by Winge (1927) in *Tragopogon* hybrids where the tetraploid parts of two roots by reason of their larger cells rendered the cross-sections of the roots eccentric. The plant bearing this chimeral root was of normal appearance in the rosette stage but unfortunately died before flowering.

The second case showing a chimeral condition was a plant of *Crepis Bureniana* ($n=4$). Of the thirty roots of this plant which were examined two were tetraploid, having 16 chromosomes in all the

plates observed. Plate 54, figures *a* and *b*, shows the chromosome complexes with surrounding areas from the outer cortex in comparable regions of the diploid and tetraploid roots. Each of the chromosomes of the diploid complex could be recognized in the tetraploid and the longest one of the set could be identified four times in the best tetraploid plate. Undoubtedly there has been a doubling of the diploid set. An examination of plate 54 shows that the average size of cells and nuclei is larger in the tetraploid root.

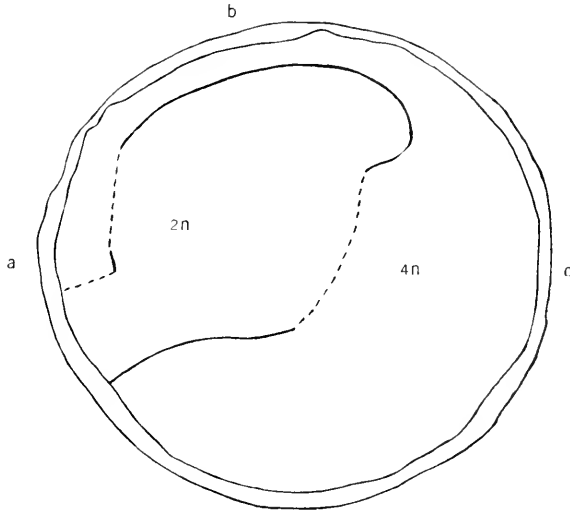


Fig. B. A cross-section of root of *biennis-setosa* hybrid derivative showing the extent of the tetraploid area. The outer ring is the extension of the root cap and contains no dividing cells.

The occurrence of two tetraploid roots in the same plant may be taken as evidence that part of the central cylinder from which branches arise had become tetraploid. It was thought possible that the plant above ground might have been affected similarly, and if so, the pollen produced on a tetraploid branch would be larger than that on a diploid. Examination of pollen from various branches, however, showed no noticeable size differences, so it was concluded that tetraploidy was probably confined to the roots.

In this same plant a root was examined which contained a number of large cells. Most of them were clearly multinucleate, from two to four nuclei having been counted in single cells (pl. 55). Plate 55 *b* shows one of the smallest of these with 2 nuclei, and 2 nuclei may be observed in one of the cells in *a*. In the larger cells nuclei were to be found in successive sections. These cells were scattered throughout

the cortex, mostly near the periphery, and in one region a group of them seems to be responsible for the somewhat misshapen appearance of the root in cross-section (pl. 55 *a,c*). The cells vary in size, sometimes extending through three or four sections 7μ thick. They are more or less vacuolated, depending on their size, but even the smallest ones could be distinguished almost at a glance by their less densely staining cytoplasm.

The normal chromosome complex of 8 was to be seen in several plates of normal cells. In one very large vacuolated cell a large number of chromosomes was observed, apparently in metaphase (pl. 55 *d*). Note the V-shaped arrangement of the metaphase plate. The chromosomes could be seen in 3 successive sections of 7μ thickness.

Nawaschin (1926) has reported a giant cell with over 500 chromosomes in a root tip of *Crepis tectorum* and takes up favorably the theory that it has arisen by successive chromosome divisions without accompanying cell divisions. The cell he shows is not greatly unlike some of those seen in this material. Here, however, there seems to be some evidence that the large cells are the result of fusion of several smaller ones. The V-shape of the one plate observed in a giant cell indicates that it may be a combination of two plates and that a nuclear fusion has taken place. Plate 55 *c* gives the impression that two large cells are in the process of fusion and indeed the cell wall has practically disappeared. It seems likely that such cell fusion might be associated with an abnormal or pathological condition of the root, further evidence of which was to be seen in small black areas probably representing degenerated cells (pl. 55 *b*).

The origin of tetraploidy in diploid tissue has been discussed by various investigators. In some cases it has been associated with specific outside influences. The effect of nareoties in inducing tetraploidy has been investigated by Nemeec (1903) and Sakamura (1920). Blakeslee and Belling (1924) found tetraploid shoots in *Datura* plants subjected to cold. Lesley (1926) found tetraploid areas in tomato plants affected by mosaic and thought it might be possible that local changes due to this disease might affect mitotic processes. Cases of polyploid cells have been attributed to abnormal processes related to degeneration as in the investing cells of the ovaries of *Anasa tristis* (Wilson, 1906). The doubling of chromosome numbers in Winkler's (1916) well-known chimeras has been attributed to the effect of wounding. Jorgensen and Crane (1927) have recently secured tetraploidy in *Solanum* by the use of Winkler's method. Winge (1927) finds that

most of the cells of the "crown galls" on sugar beets which can be induced by inoculation with *Bacterium tumefaciens* have the tetraploid chromosome number.

Nawaschin did not venture any suggestion as to causal agencies in connection with his tetraploid sector in *Crepis Dioseoridis*. Lesley believed that it was unlikely that cold played any part as a causal factor in tomato chimeras, as only the roots seemed to be affected. It has been suggested by Mr. C. W. Haney that watering greenhouse plants with cold water would provide the necessary conditions if sudden lowering of temperature has anything to do with the production of tetraploid root cells. The plants described here were entirely normal as far as could be observed and tetraploidy could not be ascribed to any special factor in the environment.

Winkler had suggested that certain tissues may regularly become polyploid and Breslawetz (1926) has reported tetraploidy as the universal condition in the dermatogen of the root tips of *Cannabis sativa*.² De Litardiere (1923) found tetraploid and octoploid cells in the dermatogen of *Spinacia oleracea*. In both these cases it would seem that the transforming of diploid into tetraploid cells must have occurred many times in the same root.

The possibility of fragmentation giving rise to these increased numbers is easily excluded in most cases. The two most favorably received theories to account for doubling are: (1) the fusion of nuclei from two cells; (2) the division of the chromosome complex without cytoplasmic division. Breslawetz has brought forward evidence that nuclear fusion gave rise to the tetraploid cells which made up the dermatogen of the roots in *Cannabis sativa*. As no diploid complexes were to be seen in that region of the root one would conclude that fusion of diploid to form tetraploid nuclei had taken place before any normal diploid divisions occurred, or at least at an early stage in the development of the root. If so, one wonders why evidences of nuclear fusion were still to be found in well developed roots. On the other hand, the paired condition of the chromosomes in some of the tetraploid cells in *Spinacia oleracea* convinced de Litardiere that these cells had just completed a chromosome division without separation of the resulting daughter chromosomes.

The occurrence of multinucleate cells in a root of the *Crepis Bureniana* plant which was partly tetraploid has been noted above. The significance of this phenomenon in the origin of the tetraploid

² De Litardiere (1924) found rare diploid cells in the periblem and in one case a tetraploid cell in the plerome of roots of this species.

roots is questionable. The presence of several nuclei in one cell and the abnormal appearance of these large cells would incline one to belittle the possibility that tetraploidy here had originated by cell and nuclear fusion. However, it has been pointed out that the one chromosome complex observed in a giant cell indicated that nuclear fusion had taken place. We cannot, therefore, dismiss the possibility that a fusion of nuclei from two cells gave rise to a cell which was tetraploid and thence to tetraploid roots. It seems more likely, however, that the occurrence of tetraploidy and of a root with giant multinucleate cells in the same plant was merely a coincidence.

Whatever the method of origin, the frequent occurrence of tetraploidy in somatic tissues throws some light on two much discussed questions. First, there is that of the mode of origin of diploid gametes. Rosenberg (1926-27), Karpechenko (1927), and others have described processes in the reduction divisions of apogamous species and interspecific hybrids by which diploid gametes are formed. The increasing frequency with which tetraploidy has been recorded in root tips makes it seem likely that it would be found in other tissues were they examined as consistently. Its occurrence in the cells of the germinal line would lead to the formation of gametes with twice the normal chromosome number. This has been shown to occur in *Datura* where tetraploid shoots were found. Presumably a smaller area might be affected and only a portion of the gametes formed on one shoot might be diploid.

In the second place, the frequent occurrence of somatic tetraploidy has a bearing on the origin of tetraploids and tetraploid hybrids. *Primula kewensis* arose as a bud sport probably from an F_1 hybrid of *P. verticillata* and *P. floribunda*. It has the sum of the diploid chromosome numbers of the parent species and Clausen and Goodspeed (1925) have suggested that it is a true tetraploid hybrid, the bud sport having arisen by a doubling of somatic chromosomes. A similar explanation with the doubling occurring immediately subsequent to fertilization was suggested by these investigators to explain the origin of a tetraploid hybrid between *Nicotiana tabacum* and *N. glutinosa*. Rosenberg (1926) has recently proposed an explanation for the origin of the tetraploid *Aegilops-Triticum* hybrid of Tschermak and Bleier (1926) which depends on the chance meeting of diploid gametes formed by a "semi-heterotypic" division. In the light of the foregoing facts it seems much simpler to suppose that a doubling of the chromosomes took place in the fertilized egg, or in some cell of the

young embryo which gave rise to the growing point of the stem. Nawasehin was led to favor this theory of the origin of tetraploids by the frequency of $4n$ plants in *Crepis tectorum*. He calculated the frequency of diploid gametes from the number of triploid plants obtained in over 4,000 individuals, and on this basis determined the number of tetraploids which should occur by chance meeting of those gametes. He found the expected number of tetraploids to be much less than that actually occurring. He concluded, therefore, that tetraploids arose through the doubling of chromosomes in the fertilized egg cells.

In view of the increasing number of cases in which tetraploidy has arisen in normal diploid tissue, one is justified in concluding that it may play a part in the origin of polyploid species and interspecific hybrids.

I acknowledge with pleasure my indebtedness to Dr. J. L. Collins and Professor E. B. Babcock for the material used in this study.

SUMMARY

Tetraploidy was observed in the roots of two different plants. One, a *C. biennis* × *C. setosa* hybrid derivative, had one root partly tetraploid. The other, a plant of *C. Bureniana*, had two roots wholly tetraploid.

No external factors could be associated with the tetraploidy.

Giant multinucleate vacuolated cells occurred in another root of the same *C. Bureniana* plant. Evidence of cell and nuclear fusion was observed. It is doubtful whether this phenomenon has any significance in relation to the origin of the tetraploid roots.

Tetraploidy arising in somatic tissue probably plays a part in the origin of polyploid species and interspecific hybrids.

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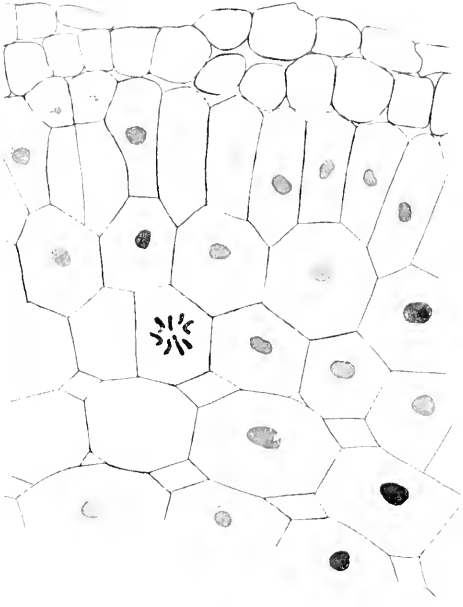
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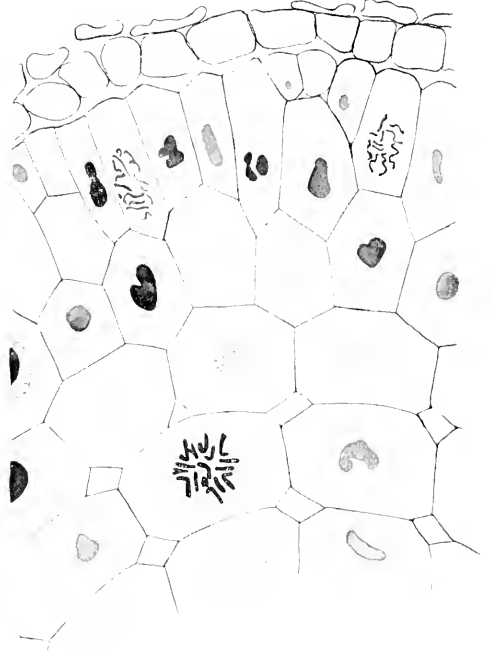
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PLATE 54

Comparable areas of *a*, diploid, *b*, tetraploid roots of a *Crepis Burreniana* plant.



a

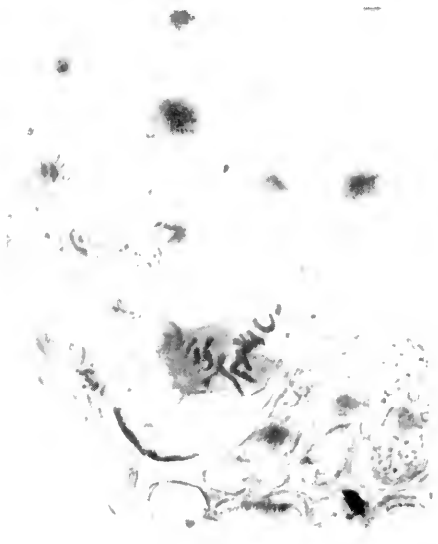
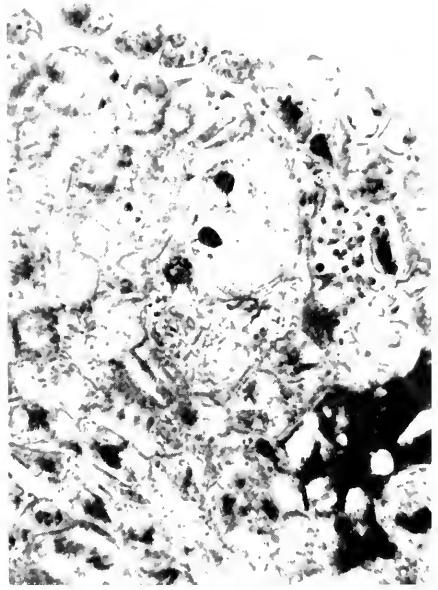
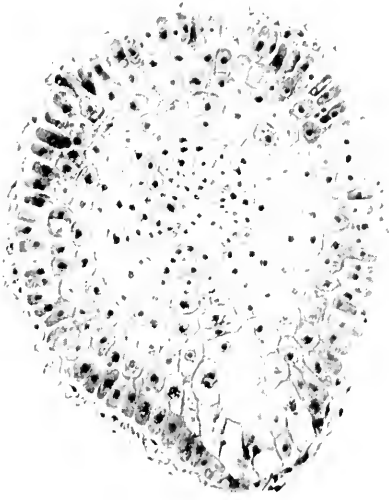


b

PLATE 55

Photomicrographs of root of the *Crepis Bureniana* plant showing giant multinucleate cells.

- a.* A cross-section showing a group of giant cells.
- b.* One of the smaller giant cells with two nuclei.
- c.* Two giant cells apparently fusing.
- d.* A large cell containing a V-shaped metaphase plate with many chromosomes.



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INTRODUCTION

The genus *Trifolium* is an outstanding genus within the family Leguminosae. It contains a large number of species which show great morphological variation and a wide geographical distribution and includes several very important agricultural crops, such as *T. pratense*, *T. repens*, *T. hybridum*, *T. incarnatum*, and *T. alexandrinum*. Although considerable plant breeding work has been carried out, especially with *T. pratense*, no genetic analysis of any of these species has been made and the cytological investigations are of very recent date. The genetic analysis of other agricultural crop plants has rendered important service to the plant breeder, and there is every reason to assume that the same will be the case with the clovers in which there are a large number of "good" genetic characters. It is of importance that the chromosome situation in these species should be known before genetic investigations are started. The results of the cytological investigations have been encouraging to the geneticist and plant breeder as they show that in the most important

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agricultural plants the chromosome numbers are fairly low—7 and 8 haploid, according to which 7 and 8 linkage groups are to be expected.

A genetic and cytological investigation in *Trifolium* was started by the writer at the Division of Genetics of the Department of Agriculture, University of California, Berkeley, in July, 1926, and carried on until December, 1927. In this paper will be included only the results of the cytological investigations and the attempts at species crossing. I take great pleasure in thanking Professor E. B. Babcock for laboratory facilities and give my best thanks to all the members of the staff in the Division of Genetics for help and advice. I am greatly indebted to Professor P. B. Kennedy and Mrs. A. Frederick, of the Division of Agronomy, for the material and for help in identification of the species used. Acknowledgment is also given to the International Education Board for the fellowship granted to me.

MATERIAL AND METHODS

Most of the material was grown from seeds furnished by Professor Kennedy. The seeds of the American species had been obtained either from plants growing wild or from plants grown one generation in the greenhouse. Plants of these species have been compared with the specimens in the Herbarium of the University of California and in the collection of Professor Kennedy. In the nomenclature and grouping of these species I have followed McDermott (1910). The other species used were for the most part well-known cultivated species, with the exception of *Trifolium glomeratum* from Syzran, Russia, and *T. subterraneanum*, which was grown only to a seedling stage. These seeds had been obtained from the United States Department of Agriculture. The two strains of *T. repens* used were obtained from the following sources: (1) *T. repens* var. *sylvestre*, wild white clover, plants growing wild on the campus of the University of California; (2) *T. repens* var. *giganteum*, Ladino clover; Italian white clover; seeds from Vilmorin, France. Three strains of *T. pratense*, Italian, Late Swedish, and Canadian, were obtained from the Central Experiment Station, Ottawa, Canada.

The chromosomes were studied in somatic divisions in root tips; in two species the reduction division in the pollen mother cells was also investigated. For the root tips the fixative of S. G. Nawaschin (Karpechenko, 1927, p. 367) was always used. Buds for the study of pollen mother cells were fixed either in Flemming's medium or

Nawaschin's fixative. Most of the plates were stained with Haiden-hain's iron-haematoxylin, a few with iodine-gentian-violet (Huskins, 1927). For *Trifolium* the following procedure was found to be the best: (1) Root tips: 70 per cent alcohol; iodine (5-10 min.); gentian violet (5-10 min.); iodine (30 sec.). (2) Pollen mother cells: 70 per cent alcohol; gentian-violet (5-10 min.); iodine (30 sec.).

Attempts were made to study the reduction division in pollen mother cells by the aceto-carmin method, but with no success. It is difficult to get the anthers out of the small buds and they are filled with inclusions (starch?) which apparently prevent the absorption of the fixative. The methods of emasculation and pollination will be described in the section on "Attempts at species crossing."

CHROMOSOME NUMERS AND MORPHOLOGY

CHROMOSOME NUMBERS

Martin (1924) counted the chromosomes in *Trifolium pratense* and *T. repens* and found the number in both to be 12, haploid. Karpechenko (1925) examined the chromosomes in somatic cells—root tips—of twenty-four species and found the following series of diploid chromosome numbers:

Diploid number of chromosomes	14	16	32	48	about 80	about 130
Number of species.....	8	12	1	1	1	1

Bleier (1925) studied the reduction division in eighteen species and found the following series of haploid chromosome numbers:

Haploid number of chromosomes	7	8	9	14	16	48
Number of species.....	5	8	1(?)	2	—	2

I have obtained chromosome numbers in ten native American species with the following distribution in the groups given by McDermott (1910).

SECTION I. TRIDENTATAE

	$2n$
<i>T. obtusiflorum</i> Hook., 2 strains.....	16
<i>T. obtusiflorum</i> var. <i>majus</i> (<i>T. majus</i> Greene).....	16

SECTION II. VARIEGATAE

<i>T. variegatum</i> Nutt.	16
<i>T. wormskjoldii</i> Lehm.	48(?)*

* But little material was available for fixation and the chromosomes were much crowded in the cells, so that the number could not be obtained with certainty. There are in figure 1c 47 bodies, one of which probably represents 2 chromosomes; 48 is very probably the correct number of chromosomes present.

SECTION III. MONANTHEAE

SECTION IV. CYATHIFERAE

<i>T. microcephalum</i> Pursh.	16
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SECTION V. VESICULEAE

<i>T. fucatum</i> Lindl.	16
<i>T. fucatum</i> var. <i>virescens</i> (<i>T. virescens</i> Greene).....	16

SECTION VI. BRACTEOLATEAE

SECTION VII. MACREAE

	$2n$	
<i>T. albopurpureum</i> T. and G.	16	
<i>T. dichotomum</i> H. and A.	32	

SECTION VIII. LONGIFOLEAE

<i>T. reflexum</i> L.	16
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SECTION IX. CILIATAE

<i>T. ciliatum</i> Benth. (<i>T. ciliatum</i> Nutt.).....	16
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The other species counted are:

	$2n$		$2n$		n
<i>T. pratense</i>	14	Karpechenko	14	Bleier	7
<i>T. incarnatum</i>	14	Karpechenko	14	Bleier	7 and 8
<i>T. repens</i> , 2 varieties....	32	Karpechenko	32	Bleier	14
<i>T. hybridum</i>	16	Karpechenko	16	Bleier	8
<i>T. glomeratum</i>	16			Bleier	7
<i>T. minus</i> (?)*	32			Bleier	14
<i>T. subterraneum</i>	16				
<i>T. alexandrinum</i>	16				

* The identification of this species is not certain, as it was not observed in flower. *T. minus* ought to be studied anew to ascertain whether it has 28 chromosomes as found by Bleier or 32 as found by me. If 28 is correct, this represents the only double species of the 7 series.

The eighteen species counted by the writer form the following series of diploid chromosome numbers:

Diploid number of chromosomes.....	14	16	32	48
Number of species	2	12	3	1

In *Trifolium incarnatum*, Karpechenko found 14 somatic chromosomes and Bleier has plates with both 7 and 8 bivalents at heterotypic metaphase. I found the somatic number to be 14, which is probably correct for this species. In *T. repens*, Karpechenko found 32 diploid chromosomes and Bleier found 14 bivalents at first metaphase. Erith (1924) counted the chromosome numbers in three varieties of *T. repens*. On page 113, Erith states, "the two cultivated races of white clover have the same number of chromosomes as the small wild

species." On page 92 it is stated, "The diploid number of chromosomes is sixteen." Figure 62*b* on the same page shows, however, a heterotypic metaphase with 16 bodies, and figure 62*d* a homotypic metaphase with 16 bodies. From these figures the conclusion must be drawn that the forms investigated by Erith had 32 and not 16 as the diploid number. I found the somatic number to be 32 in two varieties of this species. In *T. montanum*, Bleier found 9 bivalents at first metaphase; the count was not certain and as Karpechenko found the diploid number in *montanum* to be 16, it is probable that there is no species of *Trifolium* with 18 as the diploid number. There is now established the following series of haploid chromosome numbers in forty-three species of *Trifolium*:

Haploid number of chromosomes....	7	8	14	16	24	about 48	about 130
Number of species	11	23	1	3	2	2	1

The basic numbers of this series are 7 and 8. The 7-series consists of single, double, and possibly higher multiple numbers; the 8-series of single, double, triple, and probably higher multiples. This is the terminology suggested by Belling (1927); the term single is used for the species with the basic number, and double and triple for species with two and three times this number, corresponding to the old terms tetraploid and hexaploid. As for the relation between chromosome numbers and the systematic classification of species, Karpechenko (1925) states: "Hence it is evident that in the process of divergence of species of clover certain chromosome changes, undiscerned by observation, have greater significance, whereas the number of chromosomes plays a subordinate rôle." The species studied by the writer give evidence in the same direction. Widely different species, such as *Trifolium variegatum* and *T. reflexum* have the same number of chromosomes, while in one group are found species with 16 and 14 chromosomes. Among the American species studied there is no representative of the 7-series. These species form a regular multiple series, 8-16-24.

VARIATIONS IN CHROMOSOME SIZE IN THE GENUS

The chromosomes in *Trifolium* are in general small. There is, however, a very large range of variation in length from about 1 μ in *T. variegatum* (fig. 1*d*) to 4 μ in *T. reflexum* (fig. 1*b*). There is a still greater difference in total chromosome volume, as illustrated by the complexes of *T. variegatum* (fig. 1*d*) and *T. dichotomum* (fig. 1*k*).

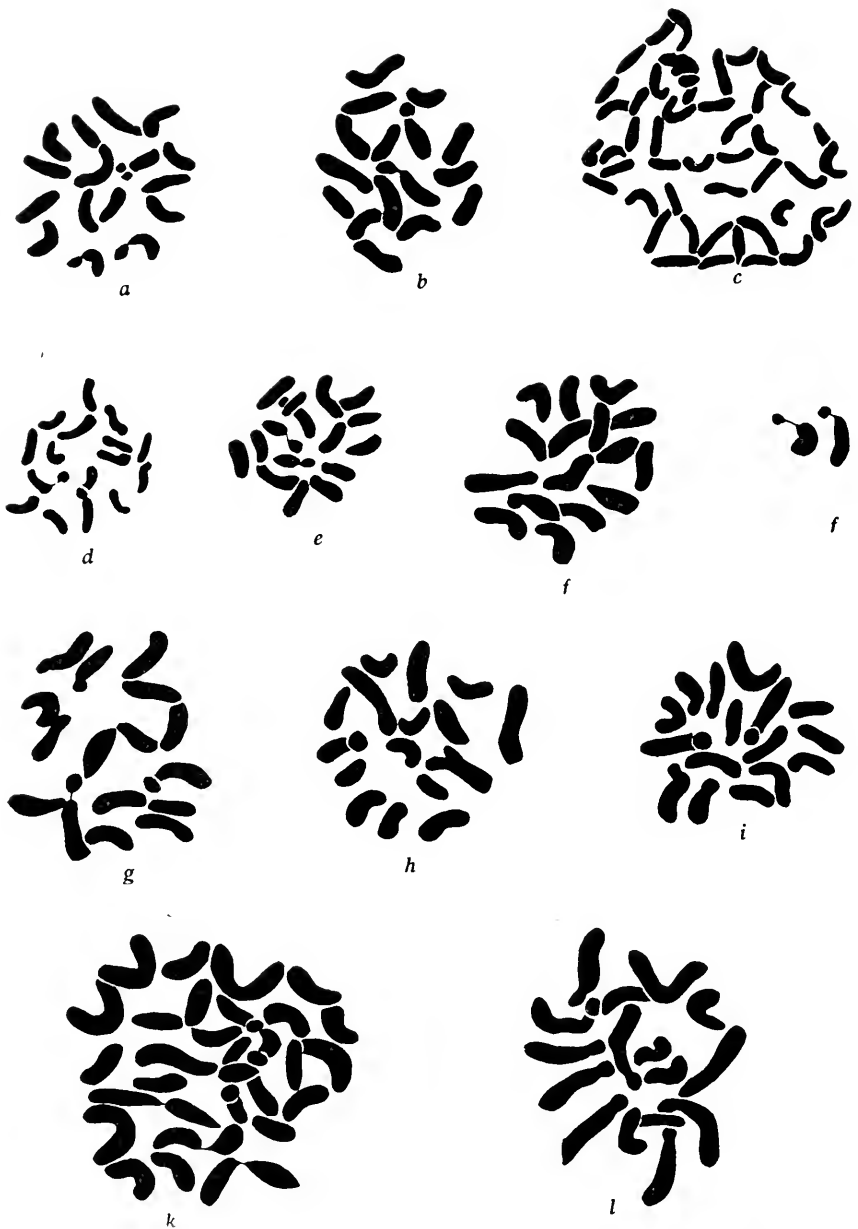


Fig. 1. Somatic metaphase figures from root tips of: a, *T. obtusiflorum*; b, *T. majus*; c, *T. wormskjoldii*; d, *T. variegatum*; e, *T. microcephalum*; f, *T. fucatum*; to the left a plate with 16 chromosomes, to the right a satellited pair from another plate; g, *T. virescens*; h, *T. albopurpureum*; i, *T. ciliolatum*; k, *T. dichotomum*; l, *T. reflexum*. All drawings for this paper were made with the aid of a camera lucida with a Zeiss 18 compensating ocular and a Leitz apochromatic 2 mm. objective, N.A. 1.3; magnification 3650; figures not reduced; sections 7μ , stained with Haidehain's haematoxylin.

The species can be grouped as follows according to chromosome size, the species in each group being arranged according to increasing size of the chromosomes:

SMALL	MEDIUM	LARGE
1. <i>T. variegatum</i>	5. <i>T. microcephalum</i>	18. <i>T. incarnatum</i>
2. <i>T. repens</i> var. <i>sylvestre</i>	6. <i>T. obtusiflorum</i>	19. <i>T. dichotomum</i>
3. <i>T. minus</i> (?)	7. <i>T. glomeratum</i>	20. <i>T. reflexum</i>
4. <i>T. wormskjoldii</i>	8. <i>T. pratense</i>	
	9. <i>T. subterraneum</i>	
	10. <i>T. albopurpureum</i>	
	11. <i>T. majus</i>	
	12. <i>T. alexandrinum</i>	
	13. <i>T. repens</i> var. <i>giganteum</i>	
	14. <i>T. ciliolatum</i>	
	15. <i>T. hybridum</i>	
	16. <i>T. virescens</i>	
	17. <i>T. fucatum</i>	

These groups are not sharply set apart; if all the chromosomes in all the complexes are arranged according to size they will form a continuous series, but if one looks at the chromosome complexes as such, the complexes in the small groups are distinctly smaller, and those in the larger group larger, than the complexes in the medium group. It is not contended that these groups have any phylogenetic significance, but they may serve to give a picture of the situation.

Bleier (1925) discusses the question of chromosome size in relation to chromosome number, nuclear size, and plant size. His discussion is based on the size of the bivalent chromosomes at the heterotypic metaphase and on measurements of the nuclear diameter of the pollen mother cells at the synaptic stage. As is pointed out by him there is great variation in the size of the metaphase chromosomes within a species. The same was found in *T. alexandrinum* in which a large number of metaphase plates were studied. In the same way the chromosomes at the somatic metaphase show some variation within the species (see figs. 2a and 2b of *T. pratense*), but the variation is less than in the pollen mother cells. Bleier makes the following statements regarding chromosome size in *Trifolium*:

1. Species with the same number of chromosomes have chromosomes of different size.
2. The nuclear volume is not dependent upon the number of chromosomes, but on the mass of chromatic substance.
3. There is no correlation between chromosome number and plant size, but species with a larger nuclear volume have larger growth than species with small volumes.

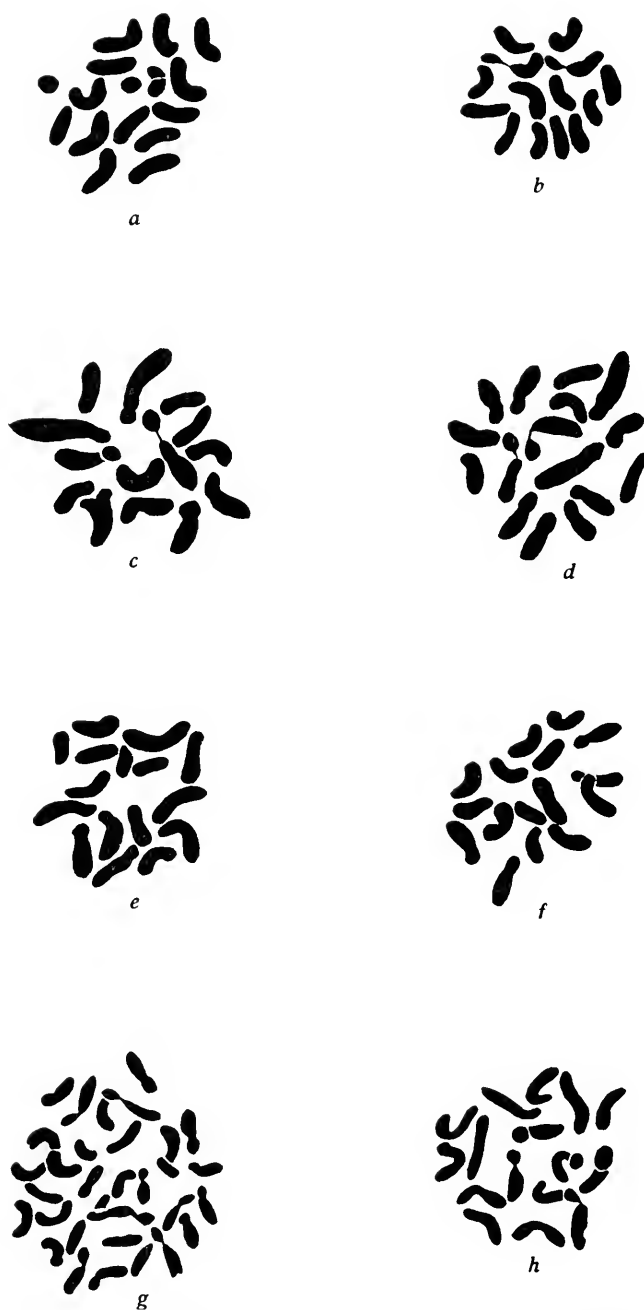


Fig. 2. Somatic metaphase figures from root tips of: a, *T. pratense*; b, *T. pratense*; e, *T. incarnatum*; d, *T. alexandrinum*; c, *T. hybridum*; f, *T. subterraneum*; g, *T. minus*; h, *T. glomeratum*. Figure 2b was stained with gentian-violet, the others with Haidenhain's haematoxylin.

The first statement is well illustrated by a comparison of the chromosomes in *T. variegatum* (fig. 1*d*) and *T. reflexum* (fig. 1*l*). The third statement may hold as a general rule, but a rule to which there are many exceptions. *T. obtusiflorum* (fig. 1*a*) has 16 medium to small chromosomes, *T. reflexum*, 16 large, but the former has the largest plant size. That one must be careful in conclusions based on comparisons of chromosome size in species of the same genus is also brought out by the cases of intraspecific variability in shape and size of chromosomes discussed below.

VARIATIONS IN CHROMOSOME SIZE WITHIN THE SPECIES

In *Trifolium repens*, two varieties were examined cytologically, *T. repens* var. *sylvestre*, wild white clover, and *T. repens* var. *giganteum*, Italian white clover or Lodi clover, three plants being studied in each variety. The three plants of *giganteum* all had chromosomes of about the same size (fig. 3*a*, which is a metaphase plate from a root tip of plant 13*a*). Of the three *sylvestre* plants, plant 1*a* showed very small chromosomes, 1*b* and 1*c* somewhat larger, but all considerably smaller than the chromosomes of 13*a* (fig. 3*b, c, d*). Karpechenko (1925) studied the somatic metaphase in *T. repens*; he makes no statement as to the variety used but his figure shows chromosomes of the same size as those found in *giganteum*. Bleier (1925) and Erith (1924) both studied pollen mother cells of *repens*. Bleier says nothing about which variety was studied, Erith states that she counted *giganteum*, *hollandicum*, and *sylvestre*, but does not say anything about differences in chromosome size, and it is not clear from which variety her figures are taken. However, when the bivalent chromosomes in her plates (1924, p. 92, $\times 1750$) are compared with those of Bleier (1925, p. 618, $\times 2150$) it is clear that the chromosomes pictured by him are at least three times as large as those of Erith.

This case is very interesting because *giganteum* with the large chromosomes is a giant variety, *sylvestre* a small variety. Erith (1924) has given detailed morphological descriptions of the two varieties which correspond to the plants used by the writer. The length and breadth of the terminal leaflet in several plants of each variety were measured and the measurements for the plants studied cytologically are given below. The figures represent the average of ten measurements.

Variety	Plant No.	Leaf size in mm.	
		Length	Breadth
<i>giganteum</i>	13a	44.1	32.4
<i>sylvestre</i>	1a	17.5	13.8
	1b	12.7	10.2
	1c	11.4	10.5

In agreement with the results of Erith, I found no difference in flower size in the two varieties. Apparently the increase in size in *giganteum* is only in the vegetative parts. In accordance with this is the fact that the pollen is about the same size in the two varieties, whereas the cells of the roots are considerably larger in *giganteum*. The same was found to hold true for the stolons by Erith (1924, pp. 110–111) who states, “In older plants the stolons of *giganteum* have a diameter two to three times that of *sylvestre*, the larger dimensions of the former being due to a greater number of individually larger cells.”

The origin of *giganteum* is not known, but very likely it arose from *sylvestre*. The genetic relations of the two varieties have not been determined, but some study has been given to chromosome size in F_1 hybrids. Plant 1a of *sylvestre* was crossed with plant 13a of *giganteum* with 1a as the mother plant. The F_1 plants are still too young to make possible any conclusion as to the behavior of plant size in this cross. Two somatic metaphases from F_1 are pictured in figure 3e and f. The chromosome size is intermediate, being nearer to that of the *giganteum* parent. This result suggests that the case may be one of Mendelian inheritance of chromosome size. Mendelian inheritance of a chromosomal character has been recorded by Lesley and Frost (1927) in *Matthiola*, in which they found that one Mendelian factor was responsible for the difference in shape of the metaphase chromosomes of the first meiotic division. Because of lack of material of the variety *sylvestre*, more work must be done to complete the study of chromosome size in *T. repens*. As this species is self-sterile, all the varieties are very heterozygous, and the plants used by the writer were very variable in morphological characters. One might expect, therefore, to find many chromosome sizes. It is hoped that it will be possible to follow up this problem by further study of the parent varieties, the F_1 , and later generations. As the cytological work of the writer has been discontinued, at least for some time, it seems justifiable to give a preliminary account of it.

CHROMOSOME INDIVIDUALITY

Karpechenko (1925) states that he finds no chromosome individuality in the species examined by him. In contrast to this the species reported upon here exhibit many differences in chromosome size and shape within the haploid sets. The most striking of these is the presence of satellites attached to the chromosomes. In five



Fig. 3. Variations in chromosome size in *T. repens*. Somatic metaphase figures from root tips of: *a*, var. *giganteum*, plant no. 13*a*; *b*, var. *sylvestre*, plant no. 1*a*; *c* and *d*, of plants 1*b* and 1*c* of the same variety; *e*, and *f*, from F_1 of the cross 1*a* \times 13*a*. Stained with Haidenhain's haematoxylin.

American species, representing three sections of the genus; and in four European species, also from three sections, there is 1 pair of satellited chromosomes. In one species, *Trifolium minus*, there are probably 3 pairs; in *T. repens* 1 pair of satellited chromosomes was seen in one plate only (fig. 3*a*). Although large, the satellites in *Trifolium* are often difficult to observe, because the chromosomes have a tendency to stick together end to end, and in the same way the satellites will stick to the end of the mother chromosome. This may

explain why Karpechenko did not find any satellites in *T. pratense* and *T. incarnatum*, in each of which 1 pair of conspicuous satellites was found. Of the species in which no satellites were found, only one, *T. hybridum*, has been investigated thoroughly enough to state with certainty that it does not have satellites.

The existence of satellites was first established by S. G. Nawaschin (1912) in *Galtonia*. Since that time they have been observed in many species and genera. The most outstanding works are those of M. Nawaschin (1925, 1926) on the genus *Crepis* and of Taylor (1924, 1925, 1926) on *Crepis*, *Gasteria*, *Allium*, and other genera. In the Leguminosae, satellites have been found in *Pisum*, *Lathyrus*, and *Vicia* (Nawaschin, 1925; Sveshnikova, 1927).

The satellites in *Trifolium* are large compared with those observed in most other species. *T. fucatum* (fig. 1f) seems to have smaller satellites, while *T. virescens* which is nearly related to *fucatum*, and perhaps should be regarded as a variety (fig. 1g) of this species, has large satellites. This may be a case of the same nature as that reported by Nawaschin (1926) in *Crepis dioscoridis*, in which he found strains differing in satellite size. It cannot be stated with certainty that there is a real difference in satellite size in *fucatum* and *virescens*. There is some variation in satellite size within the strains and as the chromosomes of *fucatum* were much crowded on the plates only a few observations of satellites were made in this species. In *virescens*, however, many observations were made, but satellites as small as those observed in *fucatum* were never seen.

A peculiar feature of these satellites is that they sometimes seem to lie free on the metaphase plate without any visible connection with any of the chromosomes, as shown in the metaphase plate of *T. pratense* (fig. 2a). The free satellites often have a more elongated shape, resembling very much a pair of small chromosomes. Anyone unfamiliar with the material would in such plates count 16 chromosomes in *pratense* and 18 in *alexandrinum*. In these two species the reduction divisions in the pollen mother cells were also studied. In *alexandrinum*, many plates of the first metaphase showed 8 bivalents (fig. 4a) and, in agreement with this, 8 chromosomes were found at second metaphase (fig. 4b). No trace of satellites was found at these stages. In *T. pratense*, both Bleier and Karpechenko found the haploid number to be 7 in the reduction divisions of pollen mother cells. Although only a little pollen mother-cell material of *T. pratense* was available, several good diakinesis plates showed 7 bivalents

(fig. 4c). As to the nature of the free satellites several interpretations can be given. It is possible that the fixation has failed to bring out the connecting thread which is really present; in this case the phenomenon has of course no significance. Against such an interpretation there is the fact that when the satellites appear to be free they are usually found far from any chromosome, on the outside of the plate, while the attached satellites usually lie in the middle of the plate and are connected with the chromosome by a short and rather thick thread. It may be, therefore, that the satellites sometimes become free in the living cell; in that case they may easily be lost in the mitotic division, giving rise to "sports" without the satellites.



Fig. 4. *a*, Heterotypic division in pollen mother cell of *T. alexandrinum*; *b*, homotypic division in same; *c*, diakinesis in pollen mother-cell of *T. pratense*; to the right is shown a bivalent chromosome with attached satellites from another diakinesis plate. Figure 4*a* was stained with Haidenhain's haematoxylin; figures 4*b* and *c*, with iodine-gentian-violet.

This suggests the possibility that Karpechenko worked with strains of *pratense* and *incarnatum* which lacked the satellites. This does not seem likely in the case of *pratense*, however, as satellites were found in four strains of this species. Without making any definite conclusions as to the nature of the free satellites, it seems useful to point to the situation as a possibility for geneticists and cytologists to bear in mind when working with these species.

In some of the diakinesis plates of *pratense* one bivalent was seen with a pair of small bodies attached like a pair of satellites (fig. 4*c*). The observation was made near the close of the work and no time was available to follow it up by a further study of the reduction divisions. It seems very probable, however, that this bivalent corresponds to the one pair of satellited chromosomes to be seen in figures of somatic metaphase. No instance is known to the writer in which satellites have been observed attached to bivalent chromosomes in the reduction division. The observation suggests that the maturation division in species with somatic satellited chromosomes should be studied, with

the particular aim of tracing the satellites through the meiotic stages. If this could be done it would add materially to the genetic significance of satellites, as it would show that they are not only a peculiar structure of the somatic metaphase chromosomes, but are also a part of the chromosome which is permanently differentiated out from the rest of the chromosome.

In many species of plants it has been found that certain pairs of the somatic metaphase chromosomes have definite and constant constrictions. In *Trifolium* the constrictions are not easily observed on account of the small size of the chromosomes. Some constricted pairs were established in several species, but an intensive study would probably reveal more constricted chromosomes. The constrictions are all subterminal.

In general there is not a great variation in chromosome size within the haploid sets in *Trifolium*. Some species, however, show conspicuous size differences, such as *T. alexandrinum* (fig. 2*d*), *T. incarnatum* (fig. 2*c*), *T. hybridum* (fig. 2*e*), and *T. reflexum* (fig. 1*l*). The chromosome morphology of the species was studied with two main objectives in view:

1. In order to be able to distinguish each member or at least the groups of a haploid set. This is usually done in combination with a genetic analysis, by which method it is possible to assign a particular gene to a particular chromosome.

2. In order to compare the chromosome complexes in species of the same genus and by this method to study their relationship and origin. It has now come to be used also in practical plant taxonomy to determine, in cases of doubt, whether nearly related forms should be ranked as distinct species. The first problem has been the chief concern of the present study of chromosome morphology in species which seemed the most promising from a genetic standpoint. In these species the following features of chromosome individuality have been revealed:

T. pratense:

1 pair of satellited chromosomes, 6 pairs of about equal size, without visible constrictions.

T. incarnatum:

1 pair of satellited chromosomes.

1 pair of large, constricted chromosomes.

2 pairs of medium, constricted chromosomes.

3 pairs of medium chromosomes without visible constrictions.

1 pair of small, constricted chromosomes.

T. alexandrinum:

- 1 pair of satellited chromosomes.
- 1 pair of large, constricted chromosomes.
- 3 pairs of medium, constricted chromosomes.
- 2 pairs of medium chromosomes without visible constrictions.
- 1 pair of small, constricted chromosomes.

The complexes in the last two species are similar but *alexandrinum* has one more pair of medium sized chromosomes.

T. hybridum:

- 6 pairs of large chromosomes, at least three pairs with constrictions.
- 1 pair of very small chromosomes.
- 1 pair of small, constricted chromosomes.

The smallest pair of chromosomes in *hybridum* is of the same size as the satellites of *pratense*, and the plates of *hybridum* resemble very much the plates of *pratense* in which the satellites have no visible connection with the chromosomes.

In *T. repens*, Bleier found in the first metaphase of the reduction division 4 small and 10 large bivalents. The somatic plates studied also indicate that there is one group of small and one of large chromosomes, but it is very difficult to get 32 chromosomes, all lying flat on the plate, so that nothing can be said with certainty as to the number of chromosomes in each group. It may be of interest to note that in the two nearly related species, *hybridum* and *repens*, we find in the former 2 pairs of small chromosomes and in the latter probably 4 pairs. In one plate of *repens* (fig. 3e) 1 pair of satellited chromosomes was seen, so it is probable that *repens* has satellited chromosomes. As *hybridum* has no satellites this would mean that *repens* has not simply twice the complex of *hybridum*.

Outstanding in their chromosome morphology are also *T. minus* and *T. reflexum*. It is interesting that the double species, *T. minus* (32 diploid), has probably 3 pairs of satellited chromosomes (fig. 2g) while no single species has been found with more than one pair of satellites.

T. reflexum (fig. 1l) exhibits a chromosome complex different from all other investigated species, with 5 pairs of large constricted chromosomes and 3 pairs of smaller chromosomes.

ATTEMPTS AT SPECIES CROSSING

The cases of recorded species hybrids in *Trifolium* are listed by Karpechenko (1925) and Bleier (1925). In all cases one of the parents was a species with a high chromosome number, *T. pannonicum* (130) or *T. medium* (80). Hitherto, however, no report has been given of an F_1 hybrid which has been cytologically investigated. Crosses were attempted between nine species in eighteen different combinations. The material was the same as that used for cytological investigations. The methods used were mainly two:

1. The heads were enclosed in paper bags before any flower had opened. Emasculation was performed when the head was about half developed. The flowers which were either too old or too young were cut away and in the rest of the flowers the anthers were removed with a forceps. This operation is fairly easy in the species with large flowers, but difficult in the small-flowered ones. The flowers were mostly pollinated immediately after emasculation, in some cases the next day. All the instruments used were washed in alcohol frequently during the work and only very few cases of selfing occurred.

2. Using plants of self-sterile species as mother plants, the pollen from other species was applied without emasculation to the stigma in flowers which had been bagged before opening.

Cross	Number of flowers crossed
1. <i>T. pratense</i> × <i>T. incarnatum</i>	950
2. <i>T. pratense</i> × <i>T. repens</i>	327
3. <i>T. pratense</i> × <i>T. hybridum</i>	284
4. <i>T. pratense</i> × <i>T. fucatum</i>	61
5. <i>T. pratense</i> × <i>T. virescens</i>	154
6. <i>T. pratense</i> × <i>T. obtusiflorum</i>	32
7. <i>T. repens</i> × <i>T. hybridum</i>	129
8. <i>T. repens</i> × <i>T. incarnatum</i>	20
9. <i>T. incarnatum</i> × <i>T. alexandrinum</i>	186
10. <i>T. incarnatum</i> × <i>T. reflexum</i>	26
11. <i>T. incarnatum</i> × <i>T. obtusiflorum</i>	290
12. <i>T. incarnatum</i> × <i>T. virescens</i>	92
13. <i>T. virescens</i> × <i>T. fucatum</i>	33
14. <i>T. virescens</i> × <i>T. obtusiflorum</i> ..	6
15. <i>T. virescens</i> × <i>T. reflexum</i>	—
16. <i>T. obtusiflorum</i> × <i>T. fucatum</i>	6
17. <i>T. obtusiflorum</i> × <i>T. reflexum</i>	15
18. <i>T. reflexum</i> × <i>T. ciliolatum</i>	11

With both methods the results were completely negative; a few seeds obtained by either method proved to be due to selfing. Outside of these there seemed to be no seed development at all. Below are given the combinations which were tried and the number of flowers crossed in each combination. All the crosses were made reciprocally except in 4, 8, 10, 14, 15, and 18.

In crosses 1, 2, 3, 5, 7, 9, and 11 the number of trials is large enough to allow the statement that hybrids between these species are not easily obtained.

In *pratense*, *repens*, *hybridum*, and *virescens* intraspecific crosses were made and seeds easily obtained, so the negative results are not due to faulty technique. *T. fucatum* and *T. virescens* are two very nearly related species or varieties of the same species which did not seem to cross. The number of flowers crossed is not large, but when crossing plants within *virescens* seeds were easily obtained. These results do not, of course, allow the conclusion that hybrids cannot be obtained between these species, but they suggest, in agreement with the results of other investigators, that interspecific hybrids are difficult to secure.

In case of the crosses *T. pratense* × *T. repens*, and *T. hybridum*, respectively, it was attempted, using the method described by Martin (1913), to study the behavior of the pollen of *repens* and *hybridum* on the stigma of *pratense*. Flowers of *pratense* were emasculated, pollinated immediately, and the stigmas picked out for observation after 18, 24, 48, and 72 hours. The stigmas were mounted on a slide in aceto-carmin and a slight pressure was exerted on the coverglass to flatten the stigma. The pollen both of *repens* and *hybridum* germinated readily on the stigma of *pratense*, but it was not found possible to follow the pollen tube growth through the style by Martin's method. Nothing, therefore, was ascertained as to what happened to the pollen tubes. It may be that the situation is the same as in self-sterile species of *Trifolium* in which, when selfed, the pollen will germinate, but the pollen tube growth is too slow to reach the ovary.

EVOLUTION OF THE CHROMOSOME COMPLEXES IN TRIFOLIUM

The chromosome complexes in many genera are now studied with the aim of tracing the relationship between the species and of finding the way in which the evolution of the species has proceeded. Attempts are also made to base the classification of species on chromosome morphology. For *Vicia* Sveshnikova (1927) has worked out a key based on chromosome morphology and finds that it corresponds very nearly to the key worked out by Ascherson based on external morphology. In *Trifolium* it is evident that there is no such parallelism in the differentiation of the chromosome complexes and of the external morphology of the species. Species which are far removed taxonomically and very different in their morphology have very similar chromosome complexes; for instance, the European species, *T. glomeratum*, and the Californian species, *T. obtusiflorum*. On the other hand, we find nearly related species with very different chromosome complexes. The wild red clover, *T. pratense*, is very similar to *T. medium*, but the former has 14 and the latter about 130 chromosomes. Furthermore, though the number is the same, the shape and the size of the chromosomes may be different. *T. pratense* and *T. incarnatum* are placed in the same subsection of the section, *Eulagopus*, but the chromosome complexes are very unlike. In *T. alexandrinum* and *T. incarnatum* we have two species differing in external morphology and in chromosome number (16 and 14) but very similar as regards the shape and size of the chromosomes, *alexandrinum* having an extra pair of medium sized chromosomes. The situation in *Trifolium* suggests that it will not be easy on the basis of chromosome morphology to trace the mutual relationship and origin of the species in this genus. The basis for such a study must be the possibility of establishing certain types of chromosomes, which can be identified in related species. Nawaschin's (1925) work on the genus *Crepis* is of this type. In ten species with 3, 4, and 5 pairs of chromosomes he established five types of chromosomes, one of which was a satellited chromosome. In the summary Nawaschin states, "Es wurde von mir festgestellt dass dieselbe homologischen Typen und Formen der Chromosomen in den Chromosomsätzen aller untersuchten Arten hervortreten." In *Trifolium* ten species at least have

1 pair of satellited chromosomes; but considering the fact that species from very different sections have the satellites and that, on the other hand, species with and without satellites occur in the same section, this feature does not help much in establishing any relationship between the species. It does not seem safe, either, to take chromosome size in general as an evidence of relationship, when we remember that the one species, *T. repens*, includes in itself almost the total variability in chromosome size in the genus. It is apparently only in the narrowest taxonomic groups that there is a similarity in chromosome morphology which points to common descent, and it is probably here that the study of the chromosomes may be of help to the taxonomist. Some facts pointing to this conclusion may be mentioned. *T. variegatum* in the section *Variegatae* has 16 very small chromosomes. In the same section is *T. wormskjoldii* with 48 equally small chromosomes. This suggests that these two species, in regard to their chromosomes, are more nearly related than the Californian clovers of other sections. The two nearly related forms, *T. fucatum* and *T. virescens*, have almost identical chromosome complexes. The chromosome sizes of the two related species, *T. hybridum* (16 diploid) and *T. repens* (32 diploid), indicate that the latter may have a complex which is twice that of the former.

The situation in *Trifolium* is interesting because it seems to demonstrate another type of differentiation of the chromosome complexes than is found in many other genera studied. The genera which have been most intensively investigated cytologically are those in which interspecific hybridization has been carried out. There has been, then, a preference for genera in which interspecific hybrids are fairly easily obtained, and in which such hybrids are common in nature. This has led some investigators to emphasize hybridization as the only factor in species differentiation, and it may perhaps not be out of the way to hold forth that there may be other ways of evolution of species. It seems only fair to do so in connection with this study in *Trifolium*, because all evidence suggests that hybridization has not played a dominant rôle in the differentiation of this genus. Hybrids are very rare in nature, if, indeed, ever observed, and no hybrids have been obtained in experiments. Taking into account only the external morphology of the chromosomes, in *Trifolium* no certain instance of "homologous" chromosomes in different species is known, whether in the form of one single chromosome, a group of chromosomes, or a complete haploid set. The existence of a satellited chromosome pair

in many species cannot, in the opinion of the writer, be taken as evidence in this direction. The fact that the satellited chromosome pair varies in size in the different species according to the general size of the chromosome complex should make one cautious in drawing any such conclusions and this is still clearer when the distribution of the satellited chromosomes is taken into account. In the section *Euamoria* we find *T. hybridum* without satellites, and *T. glomeratum* with satellites which resemble the satellites in *T. obtusiflorum* from a very different section. We are not at all justified in concluding that *glomeratum* and *obtusiflorum* have obtained their chromosome complex from a common source. In genera in which interspecific hybridization is common there have been found not only polyploid series of chromosome numbers, but all intermediate numbers as well. A typical genus of this kind is *Viola* (Clausen, 1927), which in the section *Melanium* has the following haploid numbers of chromosomes: 7, 10, 11, 12, 13, 17, 18, 20, 24, 30.

In *Trifolium* simple polyploid series without intermediate numbers are found; $2n = 16, 32, 48$; and 14, 28, (?).

It may then perhaps be justifiable to give a suggestion as to the evolution of the chromosome complexes in *Trifolium*. This genus presents a very clear demonstration of parallel variation, i.e., we find in species belonging to very different sections that evolution has proceeded along parallel lines. It seems better in accordance with the facts to ascribe this parallel variation to parallel independent mutations than to a common descent. This is supported by the fact that we find in many species similar variations from the wild type. In species from different sections is found the mutant form characterized by the absence of leaf spots. In *T. pratense* is found a variation from the normal red flower color to white; in *T. repens* a variation from white to red, but the red and white flower color in *pratense* and in *repens* is a genetically different character. The chromosome complexes show the same picture as the morphological characters. The presence of one pair of satellited chromosomes should be due, then, to independent parallel mutations and not to the fact that they have been derived from a common source. This suggestion as to the way in which the chromosome complexes in *Trifolium* have been differentiated is supported also by the variation in chromosome size described in *T. repens*, which is just an example of that kind of variation which the hypothesis supposes to take place. The great variability in chromosome morphology in *Trifolium* is held to be due,

then, to mutational changes in species isolated by interspecific sterility. It is not contended that crosses have not taken place in this genus, but it is held that the species have been thus isolated for a long time and that many mutations have occurred.

SUMMARY

1. Somatic chromosome numbers have been obtained in eighteen species of *Trifolium*, twelve of which had not been counted before; the reduction division was studied in two species.

2. The ten American species studied have the diploid numbers 16, 32, 48. No representative is found of the 7-series which is found in European species of *Trifolium*.

3. The chromosomes of *Trifolium* are in general small, but they exhibit a great variation in size, both as to single chromosomes and to total amount of chromatin.

4. In *T. repens* L. the two varieties *giganteum* and *sylvestre* proved to have chromosomes of different size. *Giganteum* is a giant variety and has large chromosomes; *sylvestre* is a small variety and has small chromosomes. F₁ plants between these two varieties showed chromosomes of intermediate size.

5. Ten species from several sections of the genus have been shown to have 1 pair of satellited chromosomes and one species probably has 3 such pairs.

6. The satellites are in some plates without visible connection with any chromosome and appear like an extra pair of small chromosomes. In a few diakinesis plates of *T. pratense* were observed bodies which must be interpreted as 1 pair of satellites attached to a bivalent chromosome.

7. On the basis of satellites, constrictions, and chromosome size, a scheme of chromosome morphology has been given for some of the species.

8. Species crosses were attempted between nine species in eighteen different combinations, but with completely negative results.

9. The suggestion is made that the diversity of chromosome complexes in *Trifolium* is a result of mutational changes in species which have become isolated by intersterility rather than the result of hybridization.

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STUDIES ON POLYPLOIDY
I. CYTOLOGICAL INVESTIGATIONS ON
TRIPLOIDY IN CREPIS

BY

M. NAVASHIN

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INTRODUCTION

Among chromosomal variations triploidy deserves first attention for several reasons. In the majority of cases it becomes a point of departure for further alterations. Except for tetraploidy, it represents the most widespread type of chromosomal variation, at least in *Crepis*. The majority of other chromosomal aberrations occur in the progeny of triploid individuals and are therefore only consequences of triploidy. Moreover, being unbalanced, a triploid individual is incapable of sexual reproduction without segregation, and this unbalance is the very source of new chromosomal combinations in subsequent generations, some of which cannot originate in any other way.

Among these derivatives from triploids should be mentioned the higher grades of polyploidy which are to be observed in the progeny of a triploid individual, as will be shown later. Some of them may be balanced and constant, the others in their turn may become sources of further chromosomal variation.

These various chromosomal variations, including the higher grades of polyploidy, produce profound changes in the biological properties of the individual, affecting, as will be seen, the viability and the tempo of development, the conditions of pollination, the relations as to interspecific crossability, etc. The influence of chromosomal variation on the size of organs and on fertility is well known.

All these consequences of triploidy, whether direct or indirect, make it an important phenomenon and one that should be kept in mind not only in theoretical work but also in practical breeding. It is sufficient here to mention the important services of trisomic and polyploid ratios in the task of localizing genes, the important results of investigations on genic balance and sex determination, etc. The value of seedless fruits and the superiority of triploid flowering bulbous plants give good illustrations of the practical importance of triploidy. Chromosomal variation also probably accounts for the origin of many new ornamental and commercial varieties.

This paper contains the first report on investigations carried on during the period 1926–1928. The investigations were conducted mainly in the laboratories and experimental grounds of the Timiriasev Federal Institute of Scientific Research and of the Comakademy in Moscow; and were completed at the Division of Genetics, University of California, under a fellowship of the International Education Board, New York.

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During the course of the work the writer had the excellent assistance of Miss Gerassimova of Moscow, which it is a pleasure to acknowledge here. The data obtained by Miss Gerassimova will be mentioned in the course of this paper. The writer is especially glad to express his gratitude to Professor E. B. Babcock and Professor R. E. Clausen, of the University of California, for many valuable suggestions and for active interest in the work.

OCCURRENCE, MORPHOLOGY, AND CYTOLOGY OF TRIPLOIDS

In a study of extensive material of *Crepis* several triploid plants, *Crepis capillaris* (L.) Wallr., *C. dioscoridis* L., and *C. tectorum* L., were found, as well as other chromosomal variations. A preliminary note on polyploidy in *Crepis* (Navashin, 1925) and general observations regarding the cytology and morphology of polyploids have already been published (Navashin, 1926). It has been shown that in populations of *C. capillaris* and *C. tectorum* triploid individuals are frequent, being sometimes present to the extent of one per cent of the total number of plants. Such a high degree of occurrence cannot be without some influence on the genetic behavior of the species involved.

Crepis capillaris is especially favorable material for study, for it is distinguished from other plants which have been investigated hitherto by its low chromosome number ($n=3$) as well as by very clear morphological features of the individual chromosomes, the latter circumstance permitting unmistakable identification even under most unfavorable conditions. This species has therefore been chosen as the principal subject of the investigations; and triploidy has been investigated less fully in two other species, chiefly for the sake of comparison with the conditions found in *C. capillaris*. As will be seen later such a comparison has proved interesting.

A single triploid plant, "1947," of *C. capillaris* was the source of the material of the present investigation; similarly a single plant of each of the other two species (*C. tectorum* and *C. dioscoridis*).¹

The somatic chromosomes of triploid plants of *C. capillaris* are shown in plate 56. As may be seen from the drawings, the homologous chromosomes in a triploid individual are present in threes instead of twos; their size and shape as well as the most minute details of their organization (the satellites and their size) are wholly unaffected under triploid conditions. The metamorphoses of the chromosomes during the prophase of mitosis differ in no way from those known to occur in a normal diploid nucleus.

Special attention should be drawn here to the remarkable relation between the satellite and the nucleolus in the prophase of the somatic division. As was first pointed out sixteen years ago by S. Navashin (1912, 1927), the satellite originally appears in the prophase on the surface of the nucleolus, and becomes attached at a certain stage of karyokinesis by a very thin thread to a particular end of a specific chromosome. The writer's observations on triploids have revealed the same phenomenon with the sole difference that three satellites are formed instead of two as in diploids. Two stages of this process of satellite formation are shown in plate 56, *a* and *b*. It should be added, however, that such observations are rather difficult, success depending entirely upon perfection of technique. The fixing fluid introduced by S. Navashin (chromic acid, formalin, and acetic acid in varied proportions), which has become popular among cytologists under various names, gives the best results inasmuch as the nucleolus becomes completely destained; on the other hand, with Flemming's solution and most of the other usual fixatives the nucleolus is as deeply stained by haematoxylin as the chromosomes themselves. It

¹ The first triploid plant of *C. dioscoridis* was found by Mrs. G. B. Medvedeva.

seems probable that the recent statements of Darlington (1926) denying the formation of the satellites on the nucleolus are merely due to unsuitable or imperfect fixation.

The somatic anaphase in a triploid plant also proceeds in a perfectly typical way (pl. 56*f*). Consequently, the whole mitotic process in triploids appears to be normal even to the smallest details; and the same is of course true also for the other two species, *C. tectorum* and *C. dioscoridis*.

The triploid plants, like all other known triploids, are distinguished by notably increased dimensions of the cells and cell organs. In these triploids the fruits and other external features are also enlarged; in fact the entire plant is somewhat larger than normal. The fertility is greatly reduced and a high percentage of the pollen is bad; all these features are well known and should be considered as characteristic consequences of triploidy. Pertinent data are presented in table 1.

TABLE 1

THE SIZES OF TRIPLOID *C. capillaris* PLANTS, THE WEIGHT OF THEIR ACHENES AND PERCENTAGE OF GOOD SEEDS, AS COMPARED WITH THE NORMAL DIPLOID SISTER PLANTS OF THE SAME SPECIES
Data of Miss Gerassimova.

	Triploids (average of 33)	Diploids (average of 11)
Height of the plants.....	88.2 cm.	69.7 cm.
Weight of 100 achenes.....	0.0439 gr.	0.0354 gr.
Percentage of good seeds.....	21.8	65.8

Besides the enlarged dimensions and reduced fertility the triploid plants were distinguished by their slow but robust growth. The majority of the triploids started to bloom about a month later than the diploid sister plants, although planted at the same time and grown under exactly the same conditions.

CYTOGENETIC BEHAVIOR OF TRIPLOIDS

As has been shown above, the triploid plant in spite of its reduced fertility sets enough seeds to produce numerous progeny. All the seeds (149) secured from open pollination of the original plant of *C. capillaris* were planted and produced 107 plants, two of which died. The chromosomal constitution of the remaining 105 plants is presented in table 2.

TABLE 2

THE CHROMOSOMAL CONSTITUTION OF THE F_1 PLANTS PRODUCED BY OPEN POLLINATION OF TRIPLOID *C. capillaris* PLANT, "1947"

Chromosomal constitution	Observed		Expected from binomial distribution	
	Number	Per cent	Number	Per cent
2n	70	66.7	13,125	12.5
2n + I	2	1.9	39,375	37.5
2n + I + I	39,375	37.5
3n	33	31.4	13,125	12.5
Total	105	100.0	105,000	100.0

The F_1 plants were further investigated. From each of them a sample of 100 open-pollinated seeds was taken and the root tips secured after planting were studied cytologically. The chromosome constitution of the F_2 is tabulated in table 3. The figures of this table, as may easily be seen, are essentially equivalent to those presented in table 2. As with F_1 , the F_2 consists of a great majority of diploids and triploids (888 diploids and triploids out of the total 959 plants) and only a negligible number (29 out of 959, i.e., about 3 per cent) of simple and double trisomies. It differs from F_1 only in the presence of a certain number of tetrasomies, tetraploids, and a single 7n plant.

In order to get data more nearly complete the triploid plants were crossed by Miss Gerassimova *inter se* and also with diploid sister plants. Many trisomic and polyploid plants were obtained from these crosses and were carefully studied. The data concerning these experiments will be reported in a later paper.

TABLE 3

CHROMOSOMAL CONSTITUTION OF F_2 PLANTS; (CF. TABLE 2)
Data of Miss Gerassimova.

Chromosomal constitution	Observed		Expected from binomial distribution	
	Number	Per cent	Number	Per cent
2n	613	63.9	119,875	12.5
2n + I	13	1.3	359,625	37.5
2n + I + I	16	1.7	359,625	37.5
3n	275	28.8	119,875	12.5
3n + I	1	0.1
3n + I + I	2	0.2
4n	38	3.9
7n	1	0.1
Total	959	100.0	959,000	100.0

Of the six theoretically possible trisomic types in *C. capillaris* five were actually obtained and grown under controlled conditions. These

types were as follows (the capital letters, *A*, *C*, and *D*, representing the three chromosome types): simple trisomies, triplo-*A* and triplo-*D* and double trisomies, triplo-*AC*, triplo-*AD* and triplo-*CD*. The expected triplo-*C* simple trisomic type has not been obtained, although each of the remaining five types was found repeatedly. Plate 57 illustrates the somatic chromosomes of the trisomic types, together with the normal diploid chromosome complex.

The trisomies are easily distinguished from the normal and triploid plants. Besides their lower viability and slower growth, they differ from diploids and triploids in the shape and color of the leaves and in a number of other features. Different chromosomal types of trisomies differ strikingly in their morphology, and can be readily recognized without cytological investigation. Some of them approach the triploid in fertility. A complete report of the investigations of these trisomic types will be given in a later paper.

The triploid plants of *C. capillaris* were finally crossed by Miss Gerassimova with normal plants of various other *Crepis* species; primarily in the hope of determining the constitution of their gametes, and also in order to test their crossability, which might differ from that of normal plants. The majority of interspecific hybrids obtained from the application of the foreign pollen to the stigmas of the triploids proved upon examination to contain a diploid chromosome complex of *C. capillaris* together with a haploid chromosome group of the other species used as the male parent; moreover, viable hybrids were obtained from crosses which had never been successful when diploid *C. capillaris* plants were used for crossing. Thus a hybrid between *C. capillaris* and *C. alpina* was obtained, a cross which had never been secured before. It has thus been shown that a triploid plant undergoes interspecific hybridization more readily than a diploid one. Finally, a hybrid plant was obtained which possessed four haploid chromosome complexes of *C. capillaris* and one of another species (*C. neglecta*). This demonstrates that triploids may produce viable tetraploid eggs in addition to haploid and diploid ones. These polyploid hybrids of various kinds proved to be much more fertile than the common diploid ones.

For comparison with the conditions discovered in *C. capillaris* the progenies of triploid plants of *C. tectorum* and *C. dioscoridis* were studied. The behavior of these species proved to be entirely different from *C. capillaris*. The majority of the plants in the progeny of triploid *C. tectorum* consisted of various trisomic types, and only a

relatively small number of diploids and triploids occurred. In *C. dioscoridis* the diploid plants predominate, and trisomies occur only in small numbers, approximately equal to the number of triploid plants.

In addition to trisomies and other variations resulting from numeric changes of whole chromosomes among the progeny of triploid plants, cases were found of alterations in the chromosomes themselves. Thus in *C. capillaris* one plant was found to possess a fragmented D-chromosome. This particular plant possessed a very small satellited chromosome (*d*), representing the proximal part of the normal satellited chromosome, which behaved as a new autonomous chromosome. Similar fragmentation of the satellited chromosome has been found to occur in the progeny of triploids in *C. tectorum*. In the latter species several other alterations in chromosome organization have also been observed (1926). One case of fragmentation is illustrated in plate 577.

Finally, several spontaneous interspecific hybrids have been found in the progeny of triploid plants. The majority of them possessed two haploid complexes of one species and one of the other; but a few were also found which possessed three haploid sets of one species and one of the other.

DISCUSSION AND CONCLUSIONS

The manner of origin of the original triploid *C. capillaris* plant ("1947") could not be finally established. Since triploidy, in contrast to tetraploidy, cannot be a consequence of a purely vegetative process, the "summation" of three haploid chromosome complexes in a zygote should theoretically be due to one of the following causes: viz., (1) formation of a diploid gamete followed by fusion with a normal haploid one; (2) dispermy; and (3) formation of an embryo from a cell of the endosperm.

In all the triploid individuals known to have arisen under experimental conditions the manner of origin has been proved only in the cases of triploid *Oenotheras* and *Daturas*, the plants being obtained artificially from crosses of tetraploids with diploids (Geerths, 1911; Blakeslee, 1924). There is no doubt, consequently, that in these particular instances triploidy is due to fusion of a diploid gamete with a haploid one. As for the other well-known instance of triploidy

found to occur spontaneously in cultures of *Oenothera Lamarkiana*, its origin still remains somewhat obscure. Based on the observations of B. Nemeec (1910) and Ishikawa (1918) on *Gagea* and *Oenothera*, R. Gates (1924) suggested that dispermy should be responsible for triploidy. The majority, however, are inclined to accept the same manner of origin as in experimental triploids, viz., occurrence of diploid gametes. The third conceivable explanation, viz., formation of the embryo from a cell of the endosperm, remains purely speculative since such a phenomenon has never been observed in any plant.

In the case of *Crepis* the writer does not hesitate to suggest the first method, for several spontaneous "triploid" hybrids have been found which have arisen through the open pollination of diploid plants of *C. capillaris* (Navashin, 1927). It is obvious that such interspecific hybrids could not obtain a diploid *capillaris* complex except from a diploid egg cell; it is quite improbable that two sperms, one belonging to *C. capillaris* and the other to another species, could fertilize a normal egg, and thus give rise to a triploid hybrid.

The occurrence of occasional functional diploid egg cells being clear enough, the question arises as to the influence of the increased chromosome number on the sexual function of the female gamete. R. Gates (*loc. cit.*) on the basis of the data on apogamous plants doubts the very possibility of fertilization of a diploid egg, the latter circumstance being, he thinks, an indirect proof of his hypothesis of dispermy. It has been shown, however, that diploid eggs are capable of fertilization as well as haploid ones; and moreover, it has been possible to demonstrate that not only diploidy but even higher grades of polyploidy do not affect the sexuality of the gamete. Pollinating triploid *capillaris* (after the usual castration) with the pollen of other *Crepis* species usually gave triploid hybrids, but, as shown above, a few plants possessing more than two haploid *capillaris* chromosome complexes were obtained. As a result of crossing two triploid plants together, not only triploid and tetraploid but also pentaploid plants were obtained. Finally, as has been shown in table 3, a heptaploid ($7n$) plant of *C. capillaris* was found in the immediate progeny of a triploid.

From these results it is evident that besides diploid egg cells triploid, tetraploid, and possibly even pentaploid or hexaploid ones are formed, and that these polyploid egg cells undergo normal fertilization, producing zygotes possessing as highly multiplied chromosome complexes as $7n$, i.e., twenty-one chromosomes instead of the

normal diploid number, six. How far increase in chromosome material may go in *Crepis* is still unknown.

These curious results clearly demonstrate once more that increase of chromatin material of itself cannot produce development of the zygote because the diploid, triploid, tetraploid, and perhaps pentaploid or hexaploid eggs are normally incapable of development without fertilization. Apparently the male gamete contributes some stimulating materials besides the usual chromatin materials. Thus direct cytological study supports once more Loeb's famous conclusions.

We may conclude, therefore, that triploidy in *Crepis* is due to the occasional formation of diploid gametes, probably of the female ones. There is a possibility, however, that an occasional diploid pollen grain may function, although it has not been certainly demonstrated.

As to the manner of origin of diploid gametes three possibilities may be suggested, viz.: (1) omission of the reduction division; (2) duplication of the reduced (haploid) nucleus; and (3) formation of tetraploid groups of somatic cells followed by normal reduction during sporogenesis in the resulting tetraploid tissues.

The data presented here being insufficient to arrive at a definite conclusion, one may equally suggest any one of these three conceivable ways, inasmuch as all of them are known to take place. Omission of the reduction division, particularly under the influence of various chemical and physical factors, is a well-known phenomenon; formation of groups of tetraploid cells, besides other numerous instances, has also been found in *Crepis* (M. Navashin, 1926; Hollingshead, 1928a); and duplication of the haploid nucleus in the female gametophyte has been recently confirmed by Newton (1927).

It is interesting to point out that cytology provides a unique method of demonstrating the mode of origin of a diploid gamete when other methods fail or for some reason are inapplicable. If the homologous chromosomes in the same nucleus possess distinguishing features one may directly arrive at a definite conclusion as to the derivation of the diploidy of the gametes, and consequently of the triploidy. Advantage may be taken of the occurrence of size differences of the satellites of homologous chromosomes, such as have been demonstrated above. If the original plant possesses unequal satellites the occasional diploid gametes produced by it will be different according to their mode of origin. Thus if diploid gametes are produced by non-reduction they will uniformly contain a pair of unequal satellites. On the other hand, if diploid gametes are formed as a result

of somatic duplication before reduction they will be of three different kinds: viz., (1) both satellites large; (2) both satellites small; and (3) unequal satellites. Finally, if duplication of a reduced nucleus has taken place, the gametes will be of two different kinds either with both satellites small or with both satellites large. If the original plant with unequal satellites is crossed with a plant having equal satellites one can easily get direct evidence as to the manner of origin of the occasional triploids. If among the triploids there are plants with three equal satellites, the cause of triploidy cannot be due to non-reduction, but if there are no plants of that sort then triploidy must be due to non-reduction. Taking special precautions one can even find out whether duplication took place before or after reduction. Special experiments in this direction are in progress.

No matter what the manner of origin of triploidy may be, it may undoubtedly play a considerable rôle in the genetic behavior of the species involved. Besides the well-known peculiarities of Mendelian inheritance caused by new trisomic and polyploid chromosome combinations, attention should be drawn here to some other consequences of triploidy.

In table 3 (p. 381) it is shown that 92.7 per cent of the entire progeny of triploid plants of *C. capillaris* consists of normal diploid plants (63.9 per cent) and triploid plants (28.8 per cent); and the remaining 7.3 per cent of higher grades of polyploidy (4.0 per cent), various trisomies (3.0 per cent), and triploid tetrasomies (0.3 per cent).

From the same table it may be seen that these figures are not in agreement with those expected from the binomial distribution of the extra haploid complex (see table 3, column "expected"), but are in conflict with it in several respects. Thus instead of the expected 75 per cent of trisomies only 3 per cent were actually obtained; and instead of 25 per cent of diploids and triploids, as a matter of fact there are 92.7 per cent. In order to explain the deficiency of trisomies the percentage of aborted seeds was determined under the supposition that they should represent elimination of eggs bearing 1 or 2 extra chromosomes. As may be seen from table 1 the triploid plants set on the average only 21.8 per cent of good seeds; the lack of 72 per cent of the expected trisomies is therefore in full agreement with the observed reduction in the number of good seeds. It is further very probable that the lack of trisomies may be due to partial zygotic sterility arising from disturbances which in the majority of instances

prevent the very formation of gametes bearing extra chromosomes; for the rare instances in which gametes are formed result in perfectly viable zygotes; simple or double trisomic individuals. On the other hand, there is a possibility of selective fertilization, although the high percentage of bad pollen in triploids makes it improbable that many pollen grains possessing extra chromosomes could exist.

Further difficulties are met with in explaining the observed numeric ratio of diploids and triploids which, as may be seen from table 3, is almost exactly equal to 2:1, instead of the expected 1:1 ratio. Although the observed ratio is similar to the usual zygotic lethal ratio, there is no reason to suggest any lethal factor of that sort, for under triploid conditions it could not give rise to the expected 1:2 ratio. A gametic lethal factor of some sort could, of course, give a 1:2 ratio, but the first following generation would immediately revert to the normal 1:1 ratio, on account of the loss of the lethal. Similarly it is impossible to suggest any stimulating factors which would make the haploid cell win in the struggle for life in the female tetrad in the young ovule. The elimination of trisomies should not, of course, affect the ratio of diploid and triploid offspring. Although it is impossible, from the purely theoretical point of view, to account for this curious ratio which occurs quite regularly in F_1 and F_2 (tables 2 and 3), two reasonable hypotheses may be suggested:

1. If the odd members of the triploid complex of *C. capillaris* are regularly distributed to the poles, the extreme variants, i.e., the haploid and diploid germ cells, will be formed in equal numbers. On the other hand, if the odd chromosomes sometimes lag, one of the sister cells of the dyad will receive an incomplete set of chromosomes instead of a full diploid complex. Cells of that sort, however, are almost incapable of further development as is evident from the absence of trisomies. It is possible that such a female dyad will give rise to a haploid embryo sac, no matter what the position of the haploid component may be, i.e., whether the haploid cell be turned toward the microphile or toward the chalaza. Such a phenomenon will cause an increase of the number of haploid eggs, and consequently of the resulting diploid plants. Similarly, lagging of chromosomes in the intermediate types of distribution may lead to an increase of the relative number of haploid gametes. Despite the reasonableness of such an explanation it still remains quite obscure why such disturbances in the reduction division should cause the observed regular ratio.

2. If one suggests the existence of a factor a double dosage of which stimulates parthenogenetic development of a diploid egg, it is very easy to explain the observed 2:1 ratio. Assuming that two homologous chromosomes of the original triploid plant contain this factor, the gametic ratio among diploid eggs will be as follows: 2 eggs bearing a simple dosage of the factor: 1 bearing a double dosage. Since the latter should develop into diploid plants without fertilization, the proportion of diploids will increase one-third at the expense of the triploids; as a consequence the ratio of diploids and triploids will be equal to 2:1, instead of 1:1. If, however, such an explanation is correct, different ratios should be expected among progenies of different triploid plants, according to their genetic constitution; plants possessing a double dosage of the "parthenogenetic" factor should give the 2:1 ratio, while those possessing a single dosage should produce diploid and triploid offspring in equal numbers. Among the 34 plants investigated 32 have not shown a 1:1 ratio but, on the contrary, the progeny of these 32 plants approximated more or less closely a 2:1 ratio; only two plants produced diploid and triploid offspring in equal numbers, the latter circumstance being perhaps merely accidental.

The reduction division being not yet completely investigated, it is impossible to say whether or not lagging of chromosomes may account for the observed ratio. On the other hand, some experimental evidence makes it probable that parthenogenetic development of diploid eggs does take place. Furthermore, it has been found that the triploid plants set a high number of good seeds if attempts are made to cross them to certain other *Crepis* species; instead of hybrids, however, these seeds produce only normal diploid *C. capillaris* plants. This phenomenon was especially striking when *C. rubra* was used as the pollen parent. The production of pure *capillaris* plants may be explained, of course, simply as an experimental error due to incomplete depollination; on the other hand, however, it seems rather strange that the progeny obtained in this way should consist only of diploid plants, while it is known that under normal conditions about one-third of the progeny always consists of triploids. It seems to be probable, therefore, that parthenogenetic development occurs possibly under the stimulating influence of the foreign pollen, which is, however, incapable of effecting fertilization. Jorgensen's observations (1928) on the parthenogenetic formation of diploid offspring in *Solanum* give a good illustration of the actual occurrence of the

process discussed above. This suggestion becomes still more probable if one recalls the occurrence of haploidy in *Crepis* recently discovered by Miss Hollingshead (1928*b*), which removes all doubt as to the possibility of parthenogenetic development of the egg in *Crepis*.

Essentially different conditions have been disclosed by Belling and Blakeslee (1922) in triploid *Daturas*. The majority of the progeny of triploids consists of simple and double trisomies together with diploids, the latter being present only in the amount of about one-third of the total number of plants, and triploid plants do not occur at all. As for the other expected combinations as well as the higher grades of polyploidy (excepting tetraploidy), they were not observed in the triploid progenies in *Datura* at all.

In triploid *Oenothera* (*Lamarckiana* var. *semigigas*) according to van Overeem (1920) the formation of all possible chromosomal combinations has been observed in ratios approximating those expected from the binomial distribution.

In triploid tomatoes according to J. W. Lesley (1928) only single, double, and triple trisomies occur, altogether amounting to about 85 per cent of the progeny; the remaining 15 per cent consisting of normal diploid plants. No tetraploids or higher grades of polyploidy were observed.

In other *Crepis* species, as shown above, the conditions are different from those existing in *C. capillaris*. In *C. tectorum* triploids behave like those of *Oenothera*; in *C. dioscoridis* they produce few trisomies and few triploids, the great majority of the offspring being normal diploid plants.

It is clear, therefore, that triploid representatives of different species, even though closely related, may differ strikingly in the types of progeny which they produce.

As respects the biological importance of triploidy, attention should be drawn first to the manifold instances of chromosomal variation which are known to occur in the progeny of triploid plants. As has been shown above, in addition to tetraploids, which are known to occur in a number of other species, pentaploid and even heptaploid plants are produced in the progeny of triploid plants of *C. capillaris*. Triploidy represents, therefore, the initial step of the accumulation of the chromatin material; how far this process can go is not yet known, but there is some evidence suggesting the possibility of far higher grades of polyploidy than those which have been found to date. Moreover, the tendency of triploids to produce polyploid interspecific

hybrids makes it probable that triploidy should be accounted one of the initial steps in the development of such *Crepis* species as *C. biennis* and *C. ciliata* both possessing about 20 pairs of chromosomes. As for the manner in which a polyploid race or a polyploid individual may become an initiator of a new species, i.e., whether mutation, hybridization, or both together may produce the specific differences involved, that is still obscure.

It may be considered as proved, however, that polyploidy in *Crepis* causes profound changes in the biological properties of the species involved. It should be emphasized here that a slowing down of the rate of ontogenetic development occurs as a consequence of polyploidy. It is perfectly clear that a delay of anthesis of only two weeks may under certain conditions give a considerable advantage to the plant. The rate of development may be affected even far more by polyploidy, a circumstance which may play a decided rôle in the invasion of an area by a new polyploid form, if this area were formerly unavailable on account of climatic conditions. If the new form is able to conquer a new territory, it will probably undergo there the influence of a series of new conditions which were absent in the original area. A result of such extension of the geographic area occupied by a given species, which might be of the first importance in the evolution of a genus, would be the meeting and consequent hybridization of species which have not previously been in contact. If the new polyploid form remains in the original area, it will nevertheless meet new opportunities of crossing with other later blooming species, due to the delay of anthesis. The foregoing suggestions apply to all weeds controlled by periods of grass cutting or time of harvest as well as to other plants independent of the agricultural activity of man. The importance, to agriculture, of the rate of development of commercial plants is of course well known.

It is of interest here to point out that the climatic frontier preventing the further advance of a given form may itself become a place of origin of polyploids due to peculiarities of temperature influences; for it is very well known from experimental investigations that unusual temperature conditions may be an important cause of the production of polyploid gametes (de Mol, 1923; Belling, 1925). After migration the polyploid forms thus produced on the limits of distribution will be isolated, the climatic barrier preventing them from meeting with the original form. The new additional polyploids eventually formed along the limits of distribution might steadily

penetrate into the barrier zone, thus continuing the process of invasion. Some evidence confirming these suggestions may be seen in *C. biennis*. This polyploid species (J. Collins and M. Mann, 1923), owing perhaps to its biennial life-cycle, has acquired the ability to inhabit an area which is unavailable to another closely related species, *C. capillaris*.

Furthermore, as was shown above, triploidy stimulates interspecific crossability, probably by reason of the higher viability of the hybrid zygote derived from a diploid egg, and the presence of a normal balanced chromosomal complex of one of the species crossed. Other features may also play a considerable part; among them the partial male sterility of triploids, which may facilitate successful cross-pollination. There is a possibility, moreover, that the structure of the stigma and of the style facilitates hybridization between some species, owing to enlarged dimensions of the cells, etc.

Such polyploid hybrids, as has been shown, are not only more viable, but, moreover, show a higher degree of fertility than normal ones; and the peculiarities of their chromosomal constitution afford favorable conditions for the production in subsequent generations of various recombinations of chromosomes of the parental species. Finally, crossing of two triploid individuals belonging to different species may produce directly a balanced amphidiploid (M. Navashin, 1927) hybrid, as a result of the meeting of two diploid gametes. The results of investigations on polyploid interspecific hybrids in *Crepis* will be reported in another paper.

The other combinatory alterations of the cell nucleus occurring in the progeny of triploids, i.e., trisomies and tetrasomies of various kinds, could hardly play any part in species formation. For *Datura* there is even direct evidence against such a suggestion, for balanced tetrasomic types show greatly reduced viability, and it is unreasonable to ascribe to them the rôle of originators of new forms. In *Crepis*, as a matter of fact, such types do not even occur.

For the purpose of genetic analysis the trisomies in *Crepis* are of the highest interest because of the fact that the individual chromosomes may be easily identified. It should be pointed out that the excellent morphological features of the chromosomes in *Crepis* allow a visual verification of the hypothesis of the origin of secondaries, the latter being supposed according to Belling (1924) to have duplicated ends of certain chromosomes. A careful study of trisomies in *Crepis* shows, however, that none of them possess even the slightest abnor-

malities in chromosome organization, not to mention duplication of the ends of the chromosomes. One must conclude, therefore, that either there are no duplicational secondaries in *Crepis* or their nature should be explained in some other way. It is hoped that further genetic study on trisomies will throw light on this question.

The frequencies of different trisomic types is not the same. In *C. capillaris* apparently the triplo-D type (possessing one satellited chromosome extra) is the most common one, while the triplo-A type occurs less frequently. Finally, the third simple trisomic type, triplo-C, probably occurs extremely rarely, if at all; at least, it was not found although numerous trisomies of the other two types were discovered. Consequently, *C. capillaris* exhibits some analogy in this respect to *Drosophila melanogaster*; for according to Bridges (1923) individuals with extra II or III chromosomes do not occur in the progeny of triploid females but triplo-X and triplo-IV flies are fairly common. The lack of these combinations in *Drosophila* is reasonably explained by the disturbances produced by the unbalanced excess of large chromosomes; in the case of *Crepis*, however, such an explanation cannot be maintained, for here the individuals possessing the smallest chromosome in excess are lacking. Evidently the reason for the absence of certain chromosomal types does not depend entirely upon the relative amount of chromatin present.

Finally, as was shown above, alterations occur in the organization of the chromosomes themselves in the progeny of triploids. The most interesting variation of that sort is the fragmentation of the satellited chromosome, the latter phenomenon leading to a formation of one very short satellited chromosome (cf. pl. 571) and another longer one, instead of the original single long satellited chromosome. This particular alteration has been found to be the most common one among other instances of reorganization of chromosomes and has been discovered in two species (*C. capillaris* and *C. tectorum*). The relative dimensions of both fragments are different in different individuals; consequently the writer's original suggestion (1926) that there should be a certain "point" in the chromosome where the fragmentation takes place, must be considered incorrect. The cause of this fragmentation is still obscure; perhaps it is in some way connected with the peculiar behavior of the satellited chromosomes which, as was shown above, are represented in the earlier stages of division by two disconnected parts (the satellite and the body of the chromosome itself) and which are also subject to striking changes in hybrid nuclei

(M. Navashin, 1927). At any rate, there is no doubt that the unbalanced conditions of the nucleus in some way stimulate chromosome alteration. Although the viability of individuals possessing fragmented chromosomes is greatly reduced, there is still some probability that fragmentation of some sort may take part in species formation. In this connection one may refer especially to *C. parviflora*, which possesses an extremely short satellited chromosome and another longer one, both suggesting in a way the products of fragmentation of the usual satellited chromosomes of *C. capillaris* or *C. tectorum*. It is of interest to note that the same process of fragmentation has also been reported in progenies of triploid tomatoes by J. W. Lesley (*loc. cit.*).

SUMMARY

1. Triploid individuals have been found in many populations of *Crepis capillaris*, *C. tectorum*, and *C. dioscoridis*, in some instances in proportions up to one per cent of the total number of plants. Such a high degree of occurrence cannot fail to influence the biological behavior of the species involved.

2. Triploid individuals differ morphologically from normal ones only in quantitative features; viz., in enlarged general dimensions and organs, in enlarged cells and cell organs, in increased size of fruits, etc.

3. The tempo of development of the triploid plants is significantly delayed, although the viability is not perceptibly reduced.

4. The fertility of triploids is greatly reduced; triploid plants of *C. capillaris* produce on the average only about 21.8 per cent of good seeds, because of failure of the majority of ovules to function. Similarly the majority of pollen grains are aborted.

5. Cytological investigations of triploids has shown that their chromosomes do not differ in any way from those of diploids except they are present in triple number.

6. The first and second generations obtained from the original triploid *C. capillaris* plant have been investigated cytologically, the total number of plants examined exceeding 1000. In *C. capillaris* 92.7 per cent of all the progeny of triploids consists of diploids (63.9 per cent) and triploids (28.8 per cent); the remaining 7.3 per cent

comprises simple and double trisomies (3.0 per cent), triploid tetrasomies (0.3 per cent), and higher grades of polyploidy (4.0 per cent). The cause of these curious ratios is still unknown.

7. Of the six possible trisomic types in *C. capillaris* five have actually been found and grown under controlled conditions. These different trisomies differ morphologically from one another; some of them are usually not less fertile than the triploid plants.

8. The other two species (*C. tectorum* and *C. dioscoridis*) behave in quite a different way. The progeny of triploid *tectorum* plants consists of a majority of trisomies, the diploid and triploid plants being present in the minority. In *C. dioscoridis*, on the other hand, the progeny of triploids contains only a small percentage of trisomies and triploids; the great majority of the offspring are diploid.

9. Crossing of triploid plants with other *Crepis* species has shown that the triploid condition in the female parent is favorable for interspecific hybridization. From these crosses certain hybrids have been obtained which could never be obtained heretofore by using normal diploid plants. Many of the hybrids thus secured were polyploid. These properties of triploids under natural conditions may play a considerable part in species formation; they also make triploids favorable material for theoretical genetic work as well as for practical breeding.

10. The formation of diploid gametes (most likely of female ones) should be considered as the original source of triploidy in *Crepis*.

11. In a number of instances the occurrence of triploid, tetraploid, and possibly pentaploid and hexaploid eggs has been proved; these polyploid gametes are perfectly viable and are capable of fertilization.

12. Triploidy in *Crepis* may play a considerable although indirect part in formation of new species, for it gives rise in subsequent generations to a number of further chromosomal variations, especially of higher grades of polyploidy. Through change in the rate of development, a polyploid individual may acquire the ability of withstanding different climatic conditions. As a consequence it may penetrate into a new territory where it may become subject to a series of various influences, especially to crossing with other species and forms. On the other hand, the climatic barrier may be considered as a possible source of new polyploid forms arising in response to extreme temperature conditions; and at the same time this barrier will provide perfect isolation.

13. It is difficult to imagine how the resulting trisomic types can play any rôle in species formation, although they represent first-class material for genetic analysis, thanks to the clear morphological differences of the chromosomes. There is some probability, however, that fragmentation of chromosomes may take part in the formation of certain *Crepis* species.

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EXPLANATION OF PLATES

PLATE 56

Somatic chromosomes of triploid plants of *Crepis capillaris*. $\times 3650$.

a, Prophase showing the satellites on the surface of the nucleolus.

b, Later prophase showing the attachment of satellites to chromosomes.

c, Metaphase showing the chromosomes of the original triploid plant "1947." Note the differences in sizes of the satellites.

d, Chromosomes of a F_1 plant possessing a set of satellites identical to the original plant.

e, Chromosomes of another F_1 plant possessing two small and one large satellites.

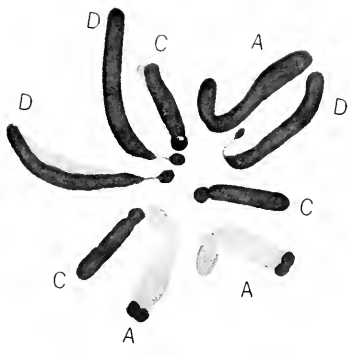
f, Early anaphase showing the regular splitting of the chromosomes and of the satellites.



a



b



c



d



e



f

PLATE 57

Somatic chromosomes of different derivatives produced by a triploid *C. capillaris* plant compared with a normal diploid chromosome complex (the capital letters representing the three chromosome types: *A*, the largest chromosome, *C*, the smallest chromosome, and *D*, the satellited chromosome). $\times 3650$.

g, Triplo-A (simple trisomic).

h, Triplo-D (simple trisomic).

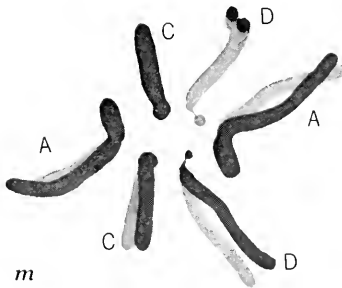
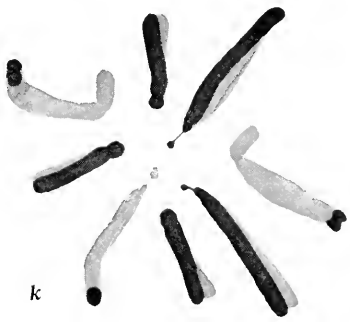
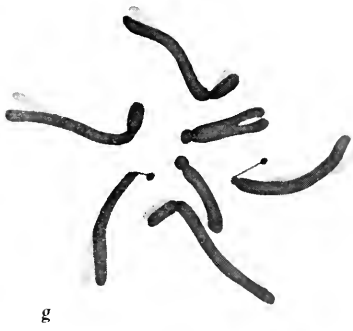
i, Triplo-AC (double trisomic).

j, Triplo-AD (double trisomic).

k, Triplo-CD (double trisomic).

l, Chromosomes of a triploid plant possessing an additional proximal fragment of the satellited chromosome.

m, Chromosome complex of a normal diploid plant.



MEIOSIS IN TWO SPECIES AND THREE
HYBRIDS OF CREPIS AND ITS BEARING
ON TAXONOMIC RELATIONSHIP

BY

E. B. BABCOCK AND J. CLAUSEN

UNIVERSITY OF CALIFORNIA PUBLICATIONS IN AGRICULTURAL SCIENCES

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INTRODUCTION

During the last few years certain species of *Crepis* have been used rather widely in cytological investigations and to some extent in genetical investigations as well. This has been due mainly to the low number and the well marked individuality of the chromosomes in the somatic nuclear plates. *Crepis* in these respects is a unique genus, especially because the parentage of spontaneous hybrids in several cases can be determined by the morphology of the chromosomes, as has been shown by M. Navashin (1926, 1927*a*, *b*).

The published investigations on the chromosome numbers of *Crepis* have been mainly the work of Rosenberg (1909, 1918, 1920), of the Berkeley group, summarized by Mann (1925) and by Babcock and Lesley (1926), and of M. Navashin (1925, 1926, 1927). The last mentioned author has specialized on the morphology of the chromosomes. Others who have contributed are Juel (1905), Tahara (1910), Miss Digby (1914), and Marchal (1920).

As the chromosomes of *Crepis* are especially satisfactory for investigation during somatic divisions, most attention hitherto has been paid to this phase. Rosenberg, however, investigated the divisions during the formation of pollen mother cells; Navashin (1927*b*) made some observations on the reduction division of *C. capillaris* × *C. aspera*; while Lesley and Hollingshead have prosecuted a number of (unpublished) investigations on the reduction division of certain interspecific hybrids of *Crepis*. But no detailed study of the course of the reduction division has been previously reported.

The present study comprises the two species, *Crepis aspera* L. and *C. bursifolia* L., together with the three hybrids: *Crepis aspera* ×

C. bursifolia, *C. taraxacifolia* Thuill. \times *C. tectorum* L., and *C. aspera* \times *C. aculeata* (DC.) Boiss. The five species used for the crossings have $n=4$ chromosomes. The chromosomes in the four species are very similar in size during meiosis, except in *C. bursifolia*, where one chromosome is conspicuously shorter than the others and different from them.

The material for the present investigations was procured by the senior author, who made the taxonomic studies and wrote section 5, while the junior author made the cytological investigations and wrote the remainder of the paper. We acknowledge with gratitude our indebtedness to Mr. C. W. Haney, who made the crossings and provided observations on the fertility of the hybrids, and to Miss L. Hollingshead, who prepared the fixations of *C. taraxacifolia* \times *tectorum* and *C. aspera* \times *aculeata*. Miss Hollingshead also made pollen counts of the hybrids. The cytological investigations were carried out during the stay of the junior author at the Division of Genetics, University of California, as a Research Fellow of the International Education Board.

METHODS

The material of *Crepis taraxacifolia* \times *tectorum* and *C. aspera* \times *aculeata* was fixed in Carnoy's fluid. This material could not be used for paraffin sections and subsequent staining with Heidenhain's haematoxylin or with iodine-gentian violet because the stain disappeared from the chromosomes as soon as from the plasm, making differentiation impossible. For these hybrids Belling's iron-acetocarmine method was successfully applied by Hollingshead to *Crepis* material fixed in Carnoy in order to have reserve material for later examinations and also in order to overcome the difficulty involved in finding time enough to do all the cytological investigations during the period in which reduction division takes place.

The acetic acid of the acetocarmine causes the pollen mother cells and the chromosomes to expand, thus overcoming the contraction and collapsing commonly caused in species hybrids by the Carnoy fixation; and in many cases the acetocarmine gives a clear differentiation between chromosomes and plasm, especially when Belling's monochromatic green light is applied. For the large chromosomes of *Crepis* the differentiation gained through the acetocarmine method is sufficiently distinct to determine pairs and univalents of chromosomes, and in determining the tetrad stages it is much more distinct and

reliable than the paraffin-section method. The disadvantages of the smear method are that it wastes much material and therefore cannot be applied to plants with so few pollen mother cells in the anthers as hybrids often have; also the acetocarmine smears do not give permanent slides, thus making it difficult later on to check up the results previously obtained. The advantage of the iron-acetocarmine method is the quickness with which it can be handled. In many cases, however, the time used for fixing and preparing the sectioned slides is so small compared with the time required for examination of the slides that it does not pay to save a little time in preparing the slides, only to waste it during the examination. Sectioned material is unquestionably much more convenient for examination than smears, where disorder reigns, where seriation of stages is destroyed, and where irregularities of tapetum cells and pollen mother cells, often found in hybrids, escape observation because the general disorder makes it impossible to determine to which type of tissue a certain cell belongs.

In the hybrid *Crepis aspera* \times *bursifolia* the following combinations of fixation and staining have been compared:

FIXATIONS	STAININGS									
Carnoy (absolute alcohol-chloroform-glacial acetic-acid 24 hours)	<table style="border: none;"> <tr> <td style="border: none;">{</td> <td style="border: none;">Heidenhain's iron-haematoxylin</td> <td style="border: none;">} sec-</td> </tr> <tr> <td style="border: none;">{</td> <td style="border: none;">iodine-gentian violet</td> <td style="border: none;">} tions</td> </tr> <tr> <td style="border: none;">{</td> <td style="border: none;">iron-acetocarmine (smears)</td> <td style="border: none;">}</td> </tr> </table>	{	Heidenhain's iron-haematoxylin	} sec-	{	iodine-gentian violet	} tions	{	iron-acetocarmine (smears)	}
{	Heidenhain's iron-haematoxylin	} sec-								
{	iodine-gentian violet	} tions								
{	iron-acetocarmine (smears)	}								
Carnoy-Navashin (Carnoy 5 minutes, followed by Navashin's formalin-chromic-acetic acid 24 hours)	<table style="border: none;"> <tr> <td style="border: none;">{</td> <td style="border: none;">iodine-gentian violet-orange (sections)</td> <td style="border: none;">}</td> </tr> <tr> <td style="border: none;">{</td> <td style="border: none;">iron-acetocarmine (smears)</td> <td style="border: none;">}</td> </tr> </table>	{	iodine-gentian violet-orange (sections)	}	{	iron-acetocarmine (smears)	}			
{	iodine-gentian violet-orange (sections)	}								
{	iron-acetocarmine (smears)	}								

As a fixative, the combination of Carnoy's + Navashin's fluid is much superior to Carnoy alone, since the latter causes too great contraction and destroys too much of the finer structure. Navashin's fluid alone has too little penetrating power and results too often in insufficient fixation, except when it is applied to very small and delicate objects such as root tips. In *Viola* hybrids, furthermore, the junior author has been able to compare Navashin's fixative with the combination of Carnoy's and Navashin's fluids and the latter has proved, in a number of cases and for this material, to be much better than Carnoy alone. It preserves the fine structural details and the objects are thoroughly fixed. In *Crepis* this fixative together with the iodine-gentian violet staining gave clear indication of the inner structure of the chromosomes.

The buds are put in the Carnoy fixative and, as for *Crepis*, the tips of the involueral scales are removed in order to facilitate penetration of the fixative between the florets. The buds remain in the first fixative from three to ten minutes, when they are changed into Navashin's formalin-chromic-acetic acid. Carnoy's fluid seems to prepare the way through the tissue for the second fixative, but in the short time of application it does not dehydrate or collapse the tissue. Kihara (1924) describes a combination Carnoy-Flemming's fluid which is based on the same principle as the combination here described.

As for the stains, Heidenhain's is too little transparent for *Crepis* chromosomes which usually must be counted in side view. Both iodine-gentian violet and iron-acetocarmine (in smears) give good transparent stains, but of these two the gentian violet gives the most contrast and the clearest, most unquestionable counts. Iron-acetocarmine applied on Carnoy-fixed material does not seem to be inferior to the fresh fixed and stained smears. The Carnoy-Navashin fixative is not so adequate for iron-acetocarmine smears as the Carnoy fluid, because the material is not brittle enough to be pulled out in very small, thin pieces. Perhaps the application of absolute alcohol for some time would give the Carnoy-Navashin fixed material the consistency that is necessary for making good smears.

The best way of applying the gentian violet for *Crepis* was to omit the iodine treatment before the staining, going directly from 70 per cent alcohol to 1 per cent gentian violet in water, applied for 5 minutes. After rinsing in water the slides were put in a solution of 1 gram iodine and 1 gram iodide of potassium in 100 cc. of 70 per cent alcohol. Here they stayed for 30 to 45 seconds and thereafter they were rushed through 70 per cent, 95 per cent, and absolute alcohol to pure clove oil with some orange G. Here the differentiation took place and after washing in xylol with a few drops of absolute alcohol in it they were put in pure xylol and afterwards mounted in balsam. The differentiation is often so perfect that the plasm is totally unstained if no orange G has been applied. In sectioned material it is necessary that one be able to see the limits up and down of the section in order to be sure that the pollen mother cell has not been cut. The orange G stains the plasm sufficiently to make its structure visible and still it gives so good a contrast to the gentian-violet stained and transparent chromosomes that these are very clearly differentiated from the plasm.

In some cases, as for example in many species of *Viola*, it is impossible to stain Carnoy-fixed material with gentian violet. Also it is sometimes better to apply the iodine *before* the staining, when the objects have been fixed in Carnoy. The iodine seems to have the effect of binding the stain to the tissue. But in many cases it binds the stain too much and it is impossible to get it out of the plasm again. The use of iodine and gentian violet can be varied in many ways. The method is also very dependent upon the fixative used. Neither in *Viola* nor in *Crepis* was any case found where iodine-gentian violet failed on material fixed in Navashin's or in Carnoy-Navashin's fluid.

In order to try the effect of acetic acid, some slides were treated for half an hour with 45 per cent acetic acid. The pollen mother cells swelled somewhat. The acetic acid did not destroy the gentian violet applied for staining but the differentiation was not so good as in the slides not treated with acetic acid. Some sectioned slides were stained with iron-acetocarmine but the effect was much inferior to that of iron-acetocarmine applied on smears. There was too little differentiation between chromosomes and plasm.

The ideal method for *Crepis* seems to be to have root tips fixed in Navashin's fluid and stained with Heidenhain's haemotoxylin (Navashin 1925, 1926), to have younger buds fixed in Carnoy-Navashin and stained with iodine-gentian violet-orange G for examination of the prophases, the heterotypic, and the homotypic divisions, and to have older buds fixed in Carnoy for examination of tetrad stages in iron-acetocarmine.

MEIOSIS IN *CREPIS ASPERA* L. AND *C. BURSIFOLIA* L.

The chromosome numbers are $n=4$ in both *Crepis aspera* (Marchal 1920; Mann 1925) and *C. bursifolia* (Mann 1925). While there is not much difference in size between the *aspera* chromosomes, Mann found one chromosome in *bursifolia* considerably shorter than the others. She gives the comparative lengths as follows (determined on chromosomes in root-tip cells):

<i>C. aspera</i>	23.9	21.5	19.7	17.5
<i>C. bursifolia</i>	24.3	22.0	19.5	12.7

Except for the shortest one, the chromosomes of the two species apparently correspond in length.

During the stages of meiotic division the small chromosome pair of *C. bursifolia* can be recognized (pl. 58, figs. 12-15; pl. 59, figs.

16-18). A corresponding difference between chromosomes cannot be seen during meiosis of *C. aspera* (pl. 58, figs. 3-11). The pairing between the homologous chromosomes seems to be perfect in these two pure species, no irregularities being observed. As for the study of development of the gemini and structure of the chromosomes, certain species of *Crepis* are very favorable, the chromosomes being fairly large, of characteristic shape, and their numbers few. By using the described combination of Carnoy's and Navashin's fixatives and staining with iodine-gentian violet-orange, a very good and clear picture was obtained.

In early prophase single and rather thin threads of chromatin are seen in the nucleus (pl. 58, fig. 1). It is not a continuous spireme, as free ends are found, but even with the small number of 8 chromosomes in the cell it is difficult to determine the number of free ends, especially since the sections were differentiated considerably in order to show the structure of the chromosomes. The thread appears thickened at small intervals thus giving the appearance of a string of beads. Apparently these thickenings are real morphological units of the chromosome, namely, the chromomeres. In the stage shown in figure 1 the thread and the chromomeres probably are double, united in parallel pairs. This seems evident partly from the fact that the thread and the chromomeres are thicker here than in the early diplophase shown in plate 58, figure 2*b*, and partly because the thread in a stage a little later than figure 1 opens up and shows double in a few places (pl. 58, fig. 2*a*). The stage depicted in figure 1, therefore, is presumably to be regarded as a *zygophase* with the chromomeres from the parallel homologous chromosomes united two and two. Some places give the impression that the chromomeres are joined end to end by double threads.

In the *diplophase* four double units are seen in each of the two species (pl. 58, figs. 2-9, 12-13). To begin with, the partners of the gemini are somewhat irregularly twisted around each other several, up to 5-6, times (pl. 58, figs. 2-3). That each of these units represents a homologous *pair* of chromosomes is evident from the number, which is four. Thus the two constituent members of a given unit cannot be explained as the split halves of one chromosome but must necessarily be one of the partners of a conjugated *pair* of chromosomes. Parasynapsis is unquestionable in this case.

A little later the chromatin thread of the chromosome coils up into a more or less regular spiral, as one of the chromosome pairs in

plate 58, figure 4 clearly shows at one end; while the less clear parts of the chromosomes in this and in several other pollen mother cells appeared like a string of beads, the beads being much larger than in the earlier stages. Also the beads here apparently were not chromomeres but short spiral coils. Real spirals with tight windings, giving the impression of bars on a ribbon, might also sometimes mislead the observer.

As the diplophase proceeds, the paired chromosomes gradually uncoil and the chromosomes become shorter and thicker. This is probably due to the fact that the chromatic spiral of the chromosome, the *chromonema* as Vejdovsky, Kaufmann, and others name it, becomes larger in diameter. The four pairs of chromosomes in plate 58, figure 4 have not more than about two twists each.

In a little later phase, the typical *diaphase* (or diakinesis) each of the members contains two chromonemata or chromatids as shown in plate 58, figures 5, 8, and 13. The splitting for the homotypic division has here become visible, but the process of splitting may have taken place in an earlier phase. In several cases the spiral structure is very clear and regular, in other cases (perhaps a little later) the spirals are not so regular and tight (fig. 12). Although here the two chromonemata within one chromosome are a little twisted, we might with poorer staining get the impression of a *longitudinal split*, which is shown in so many pictures of chromosome division. This might also, in other cases, be due to the fact that the two spiral chromatids within the chromosome really have become separated from each other (Kuwada, 1926, 1927). The two zigzag spirals crossing one another within one chromosome also might sometimes give the appearance of alveolized chromosomes or of a reticulum.

As the chromosomes uncoil during the latter part of the diaphase there is often seen a connection between the two members of a chromosome pair. In several cases it can be seen that one of the spiral chromatids extends from one chromosome to its homologue while one of the chromatids from the latter chromosome extends to the former, the two connecting chromatids sometimes forming a cross where they change chromosomes (pl. 58, fig. 7). This phenomenon is very similar to the chiasmata which Janssens (1909, 1924) saw in the nuclei of insects and apparently is what Belling (1928a, p. 284) calls an X chiasma; but in *Crepis* the outline of the chromosomes is much more distinct than in Janssens' pictures, the nature of the connections, therefore, much less questionable.

The chiasmata must have been formed earlier but are not seen clearly before the two homologues uncoil. They might have been formed, as Belling suggests (1928*a*, pp. 288–290), by coincident breaks in two of the chromatids of the homologues and subsequent attachment of the wrong ends of the broken chromatids as text figure 1, *a, b*, shows. When the homologous (double) chromosomes are separated in heterotype meta-anaphase the rearranged chromonemata probably are pulled out of the chromosomes to which they originally belonged, as figure 1, *c, d*, indicates. In these figures no attention has been paid to the complication caused by the spiral twisting of the chromatids around one another or to the chromomeres.

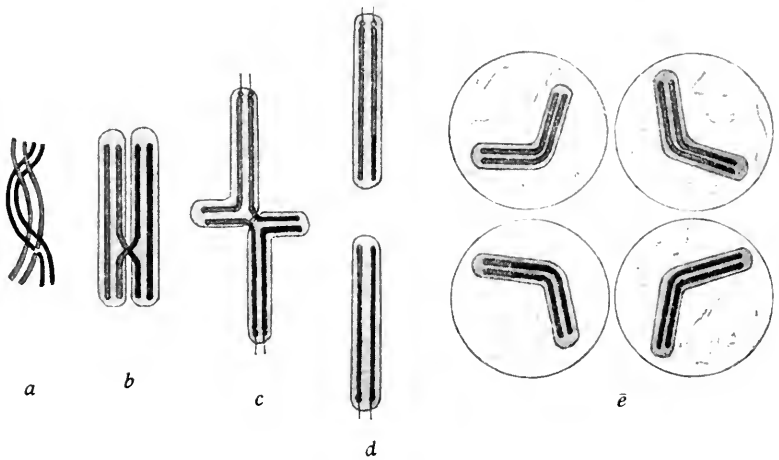


Fig. 1. Diagram illustrating the way crossing over might take place between two homologous chromosomes in the four-strand stage and chiasmata might be formed in accordance with the observations on *Crepis*.

a. The four strands twisted around each other in diplophase. A coincident break is shown in two chromatids from different homologues; the "matrix," in which the chromatids later on appear embodied, does not show in this stage, therefore the connection is more close.

b. Diaphase. The outline of the chromosomes shows up. An X-chiasma has been formed by wrong attachment of the two chromatids at the breaking-place.

c. Heterotypic metaphase; one of the two partners has turned around, a cross has been formed by pulling, but the chromosomes still have retained their individuality.

d. Heterotypic anaphase; the two chromatids with exchanged sections have been pulled out from their respective chromosomes and are enclosed within the matrix of the anaphasic chromosomes.

e. Tetrad stage; each of the four different chromatids has split longitudinally.

As will be understood, in all gemini in which chiasmata occur the X-crossed chromatids run from one chromosome to another. Which two of the four chromatids will go to either pole during anaphase will depend upon which two are together in the ends of the chromosomes

which have the fiber attachment. The disjunction of the spirally coiled and intercrossed chromatids must be a little difficult and in different organisms many anaphases show that the homologous chromosomes adhere very tightly to each other, sometimes showing a long connection between even rather widely separated homologues. It would be expected that exchanged parts of chromatids during this pulling would be straightened out and uncoiled to some extent.

The four gemini, in the diaphase of the two *Crepis* species mentioned, assume different shapes according to whether there is any connection between the two homologues or not, and according to where an eventual chiasma has taken place. So sometimes they form V's, sometimes X's, sometimes K's, and sometimes they are just parallel (pl. 58, figs. 5-9, 12-13). Figures 7 and 12 show gemini with presumably two connections (nodes, in Belling's terminology). Figure 8 shows a pair with a very conspicuous chiasma.

The shape of the bivalents during disjunction in the heterotypic meta-anaphase apparently is determined by their mode of connection during the late prophase. Plate 58, figures 10-11, shows this stage in *C. aspera*; figures 14 and 15, the corresponding ones in *C. bursifolia*. Only the small chromosome pair of *bursifolia* is rod-shaped and always very conspicuously different from the other pairs. Plate 59, figures 16-18, shows heterotypic anaphase and homotypic metaphase and anaphase of *C. bursifolia* in order to illustrate that the short chromosome of this species can be distinguished from the other three during all phases of division. During the phases following the diaphase the chromosomes stained so dark that it was impossible to see any structure in them. For the study of chromosome structure in these phases objects with less heavily staining chromosomes than those of *Crepis* should be used.

The facts observed regarding the spiral structure of the chromosomes in *Crepis* are a new link in the chain of observations made by many cytologists on different plants and animals, as for instance *Tradescantia* (Baranetzky, 1880; Kaufmann, 1926a; Kuwada and Sugimoto, 1926; Sakamura, 1927); *Ascaris* (Bonnievie, 1908; Vejdovsky, 1911-12), *Podophyllum* (Kaufmann, 1926b), *Fritillaria* (Newton, 1927), and *Vicia* (Kuwada, 1926, 1927). Also the zigzag filaments in chromosomes of *Paris* and *Listera* described by Martens (1922, 1924) might be considered evidence for a spiral structure. The occurrence of such structure very naturally explains how the chromosomes in the course of a short time change in form from long,

thin threads to short, thick rods. It also makes it more probable that the chiasmata described by several authors and hitherto mainly observed in animals may be the cytological evidence of crossing-over phenomena met with in genetical investigations. The chiasmata are observed in rather late prophases where the chromosomes are comparatively short and thick, and the fairly stable percentage of crossing over between certain genes would hardly be explained through exchange between elements so thick as the chromosomes are in this stage. But if the chiasmata are to be regarded only as evidence of an interchange or connection that has been accomplished before the chromosomes have been shortened by coiling up of the chromonemata, then it is more intelligible. Actual crossing over will then take place at an early stage but after the chromonema has been doubled to chromatids within the chromosome. The chiasmata will remain as an evidence of a crossing over that already has taken place, and the extraction of the crossed-over chromatids, finally, will not take place before the heterotypic anaphase.

Chiasmata in plants similar to those just described in *Crepis* have previously been shown to exist in *Uvularia* (Belling, 1926), *Tulipa* (Newton, 1927), and *Hyacinthus* (Belling, 1927). Newton dared not insist upon any interchange of parts of the chromatids, as the opening up of the four-strand group of chromatids in two different planes might explain the peculiar appearance. But in *Crepis* it hardly seems possible to explain the appearance of some gemini, as for instance the cross in plate 58, figure 8, in any other way than by admitting a segmental interchange between chromatids of homologous chromosomes. Here the outline of the chromosomes is seen clearly and there is no question as to which chromosome the different parts of the four chromatids belong. Thus far only one case of genetical crossing over has been observed in *Crepis* (Collins, 1924, p. 268). On the other hand *Crepis* is a favorable genus for a study of the chromosomal mechanism, and the occurrence of a haploid *C. capillaris* with only three chromosomes, and all three morphologically different, opens up a new field for a study of the early phases of meiosis, as Hollingshead (1928) has pointed out in her paper about the discovery of this haploid.

Chodat (1925) described in *Allium* a development of the heterotypic bivalent chromosomes from which he very ingeniously drew the conclusion that interchange between homologous chromosomes must take place, although in this case at the end of the chromosomes.

Not much of the structure of the chromosomes has been shown, and it is hard to be convinced that these big chromosomes really have exchanged parts at their ends, although the possibility of such exchange cannot be denied.

MEIOSIS IN THE HYBRIDS

1. *Crepis aspera* \times *C. bursifolia*.—This hybrid was more extensively studied than the other two, because living material was obtainable for new fixation in the combination of Carnoy's and Navashin's fixatives in addition to the Carnoy-fixed material procured by Hollingshead.

The affinity between the chromosomes of the two species is not strong. Although in some cases 4 pairs of chromosomes can be found during the heterotypic division, more often only 3, 2, 1, or no pairs at all are found, as will be seen from table 1. Plate 59, figures 19–22, shows that spiral chromatids are seen within the chromosomes of the hybrids just as they are seen within the chromosomes of the parents during diaphase. As shown by figures 19–20, chiasmata can also be seen in the hybrid, indicating that crossing over might be found if offspring should be procured. Attention has been paid to the fact that an unequal pair ought to be found in all pollen mother cells with 4 pairs. This unequal pair, consisting of a small *bursifolia* and a larger *aspera* chromosome, can be traced all through. According to unpublished investigations of Hollingshead the small *bursifolia* chromosome is the one with a satellite. M. Navashin (1925, p. 107) observed that the satellites were connected with the nucleolus. In accordance therewith the unequal pair in *C. aspera* \times *bursifolia*, when present as a pair, is found near the nucleolus (pl. 59, figs. 19–22). Whether the partner of the small *bursifolia* chromosome is the satellited *D*-chromosome from *C. aspera* (M. Navashin, 1927, fig. 4e) could not be settled, as the satellites do not show in the diaphase, but very probably it is that one because the pair is located at the nucleolus.

When an unequal pair is present, its partners are usually located end to end (pl. 59, figs. 19, 20, 22). This should indicate that no crossing over had taken place. Still, in a few cases there might be a possibility for a chiasma; thus in figure 21 the unequal pair seems to be parasynthetic at one end. A question which would be interesting to decide, is whether these two chromosomes unite at the satellited end or not. The chromosomes might be homologous with respect to this end.

TABLE 1
 TYPES OF MEIOSIS I IN THREE *Crepis* HYBRIDS AS MANIFESTED BY THE OBSERVED POLLEN MOTHER CELLS

Hybrids	Number of bivalents and univalents					Total Number p. m. c.	Number of irregular p. m. c.	Per cent of irregular metaphase I	Number of chromosomes detached during meiosis I			Per cent p. m. c. with detached chromosomes	
	4I	3I+1 ²		2I+4I	1I				0	1	2		3
		3I+1 ²	2I+4I										
<i>C. aspera</i> × <i>bursifolia</i>	11	24	11	8	5	59	48	81.4	155	57	14	31.4	
<i>C. taraxacifolia</i> × <i>lectorum</i>	9	8	11	2	30	21	70.0	4	9	9	(82.6)	
<i>C. aspera</i> × <i>aculeata</i>	22	11	3	36	14	38.9	47	2	4.1	

TABLE 2

IRREGULARITIES OBSERVED IN THE LATE TELOPHASE OF MEIOSIS II AND THE TETRAD PHASE IN THE THREE *Crepis* HYBRIDS
 Figures indicate number of pollen mother cells.

Hybrids	Number of nuclei in late telophase of meiosis II							Other irregularities			Total of irregular groups	Per cent of irregularities				
	4	4+1 mic.		4+2 mic.	4+3 mic.	6	7	8	9	10			Pentads	Triads	Diads	
		4+1 mic.	4+2 mic.													
<i>C. aspera</i> × <i>bursifolia</i>	39	18	13	3	3	1	2	1	2	2	1	85	46	54.1
<i>C. taraxacifolia</i> × <i>lectorum</i>	44	26	22	19	1	3	1	3	1	120	76	63.3
<i>C. aspera</i> × <i>aculeata</i>	38	2	1	2	43	5	11.6

p. m. c. = pollen mother cell; mic. = micronucleus; 4+1 mic. means 4 nuclei+1 micronucleus are observed in the pollen mother cell in question. The cases listed under 4 nuclei cover late telophases with 4 nuclei without any micronuclei and real tetrads without micronuclei and microcytes as well.

When only three pairs are present, the two univalent chromosomes usually are a short and a long one (pl. 59, fig. 24), but at least in one pollen mother cell with only three pairs, one of the pairs was unquestionably unequal (fig. 25). This shows that the affinity between the two members of the unequal pair is not much inferior to the affinity between some of the other chromosomes and it also shows that, when there is variation in number of bivalents, the chromosome pairs do not always follow the same consecutive order as regards pairing. The unequal pair shows much resemblance to an *XY* pair of sex chromosomes (pl. 59, fig. 29).

The small *bursifolia* chromosome can be recognized through heterotypic anaphase (pl. 59, fig. 30), homotypic metaphase (pl. 60, fig. 33), and anaphase (pl. 60, fig. 34). In figure 31, showing a heterotypic anaphase, it apparently is hesitating between the two poles and has split longitudinally. Unfortunately the hybrid is almost completely sterile. It sets only a few seeds by open pollination. Cytologically it would offer some advantages for a combined cytogenetic study, as the small *bursifolia* chromosomes can be followed through almost all phases of meiosis.

The most common case of pairing between the chromosomes in heterotypic metaphase is 3 bivalents + 2 univalents (table 1). Even if several univalents are present, the distribution of the chromosomes to the two poles in many cases will be 4 + 4 (table 3). This gives 4 chromosomes in most of the homotypic daughter nuclei (table 4).

TABLE 3

DISTRIBUTION OF THE CHROMOSOMES AS COUNTED FROM HETEROTYPIC ANAPHASES AND HOMOTYPIC METAPHASES IN *Crepis aspera* × *bursifolia* F₁
(3/1/4 means 3 and 4 to the two poles and one detached.)

Distribution of chromosomes	4/4	3/5	2/6	3/1/4	2/1/5	3/2/3	Total
Number of pollen mother cells	12	2	1	2	1	1	19

TABLE 4

NUMBER OF CHROMOSOMES IN HOMOTYPIC DAUGHTER NUCLEI OF
Crepis aspera × *bursifolia* F₁

Number of chromosomes	6	5	4	3	2	Total
Number of nuclei	1	3	26	6	2	38

These counts are all from fixed and sectioned material. The deviation from the 4:4 distribution in several cases is caused by a detachment of one or two chromosomes (pl. 60, figs. 31, 32).

But the homotypic division and the following "tetrad" formation cause many irregularities as plate 60, figures 35–41, shows. Table 2 tells something about the nature of these irregularities. Usually the tetrad consists of 4 cells, but these may contain more than one nucleus. In such cases the extra nucleus or the extra nuclei are very small, apparently consisting of only one chromosome (pl. 60, figs. 37, 39). Sometimes these 4 cells are of very unequal size as figure 40 shows, the tetrad containing 4 cells but 8 nuclei. A pentad is shown in figure 38 and a triad with one large and 2 smaller nuclei in figure 41. Two pollen mother cells with a tendency to form diads are shown in figures 35 and 36. They have numerous nuclei. The "tetrad" stage as a whole gives a very irregular impression. It seems as if the walls circumscribing the cells that later become pollen cells are formed regardless of the nuclei, including 1, 2, 3, or perhaps 4 nuclei as the chance may be. In this way triads and diads may be formed. Probably most of the micronuclei degenerate.

There is an increase in amount of irregularity from the end of meiosis I, giving 31.4 per cent of pollen mother cells that have one or more chromosomes detached, to the end of meiosis II where 54.1 per cent of irregularities of different kinds are found (tables 1 and 2). To this percentage must be added the cases in which the irregularity does not manifest itself in the form of extra nuclei or in other ways but consists only in an unequal distribution of the chromosomes to four daughter nuclei as for instance $5 + 5 + 3 + 3$ instead of $4 + 4 + 4 + 4$.

It is hard to account for sterility in such a case as this. Even if irregularities are very common, still about 46 per cent of the pollen mother cells consist of only 4 nuclei, and a fairly large percentage of these ought to have 4 chromosomes. And also some of the irregular pollen mother cells ought to give gametes with 4 chromosomes. If only gametes containing the same sets of chromosomes as the pure parental species are viable, then the sterility can be accounted for satisfactorily. Out of 16 gametes with 4 chromosomes one gamete ought to give a pure set of *aspera* and one a pure set of *bursifolia* chromosomes. Each floret has only one ovule and if the irregularities in the formation of the eggs are similar to those for formation of pollen there should be expected one ovule with a pure set of *aspera*

and one with a pure set of *bursifolia* chromosomes among 40 to 50 florets. If about the same amount of pollen—good and bad together—were available for each ovule, or if F_1 were backcrossed with pollen of one of the parent species, we should expect at least 4 or 5 per cent of the florets to set seed giving either pure *aspera*, pure *bursifolia*, or the F_1 hybrid type again. But among the gametes calculated to contain either *aspera* or *bursifolia* chromosomes alone, some might contain chromosomes that have been changed by crossing over, so that the balance has been disturbed. The *Crepis* chromosome groups do not seem to withstand even a small change in balance as well as do, for instance, the chromosome groups in certain *Viola* hybrids (J. Clausen, 1926). All these things ought to account for the small degree of fertility. Haney estimated the fertility to be about 3–4 per cent by open pollination. No seeds have been collected.

The type of meiosis described for this *Crepis* hybrid is dissimilar to the type described by M. Navashin (1927) for the hybrid *C. capillaris* \times *aspera*. In this last-mentioned hybrid lengthwise splitting apparently is a common phenomenon.

2. *Crepis taraxacifolia* \times *C. tectorum*.—Meiosis in this hybrid is similar in general to that in the hybrid just described. Of *C. taraxacifolia* \times *tectorum* only iron-acetocarmine smears have been used and only from material fixed in Carnoy's fluid by Hollingshead. An attempt to make permanent, sectioned slides from this fixation was unsuccessful. No differentiation could be obtained either with iodine-gentian violet or with Heidenhain's iron-haematoxylin. The iron-acetocarmine could be used but did not give good differentiation.

From table 1, page 412, it will be seen that the most common situation met with in heterotypic metaphase was 2 bivalents and 4 univalents, while 70 per cent of heterotypic metaphases are irregular. Detachment of chromosomes during meiosis I is common and there are 63 per cent of irregularities in the tetrad phase. Plate 61, figures 42–45, gives different heterotypic metaphases with from 4 to 2 pairs of chromosomes. No visible difference in size is here discernible among the chromosomes. Plate 61, figure 46, shows a regular, and figure 47, an irregular homotypic anaphase, while figure 48 is a schematic figure drawn to show how the chromosomes in figure 47 would be distributed among the nuclei a little later. Figures 49 to 54 show how the tetrad phase looks in this hybrid. Figure 49 is a nonad. As only 16 chromosomes will be present in the nine nuclei altogether, it is not hard to figure out how many chromosomes will

be in each of these nine nuclei; five of them ought to have 1 chromosome each, one would have 2 chromosomes and three ought to have 3 chromosomes each. Figure 52 shows an unquestionable diad and figures 53 and 54, triads, figure 54 having 4 nuclei but only 3 cells; figure 51 shows a tetrad with cells unequal in size. The amount of fertility is about the same in this hybrid as in the preceding one, according to Haney. Table 5 gives an analysis of 13 pollen mother cells in homotypic anaphase by direct count of chromosome number. Actually a regular distribution of the chromosomes seems to be fairly common. Table 6 shows that 66.6 per cent of the nuclei observed in these 13 pollen mother cells have 4 chromosomes. One fact must here be borne in mind, namely, that pollen mother cells with a regular distribution of the chromosomes will show up best and are more likely to be countable than those with fairly irregular distribution of chromosomes. If no crossing over took place and if 66.6 per cent of the gametes have 4 chromosomes, then 8.3 per cent of the gametes ought to have either a pure *taraxacifolia* or a pure *tectorum* group of chromosomes. The plants exhibited exceedingly low fertility but this may have been partly caused by the unfavorable season at which they reached maturity.

In this hybrid no chromosomes can be distinguished during the meiotic divisions as belonging to either of the parental species. They are all of practically the same length.

3. *Crepis aspera* \times *aculeata*.—This hybrid is conspicuously different from the first two as regards the number of irregularities during meiosis, being much more regular. As in *taraxacifolia* \times *tectorum*, the chromosomes of the two parental species cannot be distinguished during meiosis. Four bivalent chromosomes are most commonly observed in the heterotypic metaphase; only 38.9 per cent of the pollen mother cells show irregularities during this phase; and only 4.1 per cent of the pollen mother cells have chromosomes detached through meiosis I (table 1, page 412). From table 2 it will be seen that only 11.6 per cent of the pollen mother cells in the tetrad phase have irregularities. The hybrid is fairly fertile; it is estimated that 35 to 40 per cent of the florets set seed.

Plate 61, figures 55–58, shows some of the division figures, mainly of the relatively few irregular ones. Figure 57 shows a heterotypic anaphase with the distribution of 4 chromosomes to one pole, 3 to the other, and 1 chromosome between the plates, splitting. Figure 58 is a tetrad with a micronucleus in each of two of the cells.

Only iron-acetocarmine smears from Carnoy-fixed material have been used in the investigation of this hybrid, also, because no differentiation was obtainable in the imbedded and sectioned material.

TABLE 5
DISTRIBUTION OF CHROMOSOMES IN HOMOTYPIC ANAPHASE OF
C. taraxacifolia × *tectorum* F₁

Number of chromosomes in the four nuclear plates						Number of p. m. c.
A	Between the plates	A ₁	B	Between the plates	B ₁	
4	4	4	4	5
4	4	4	4	2
3	2	3	3	2	3	1
3	2	3	4	1	3	1
3	2	3	3 + 1/2	1	3 + 1/2	1
3	2	3	4	4	1
5	5	3	3	1
4	1	3	×	1	×	1
Total						13

× means that the nuclear plate in question could not be counted.
A and A₁, B and B₁ are corresponding nuclear plates.

TABLE 6
NUMBER OF CHROMOSOMES IN 48 HOMOTYPIC ANAPHASE NUCLEI AS CALCULATED
FROM TABLE 5 (DIRECT OBSERVATION)

	Actual counting			Total
	3	4	5	
Number of chromosomes	3	4	5
Number of nuclei	14	32	2	48
Per cent	29.2	66.6	4.2	100

The type of conjugation described in the *Crepis* hybrids, with a fairly wide range of variation in the amount of pairing between the chromosomes, has been observed by the junior author in a number of *Viola* hybrids. Apparently in these cases *some* affinity must exist between all the partly homologous chromosomes as they sometimes all pair. Still the affinity must be somewhat weaker as the chromosomes often fail to conjugate. These cases of partial affinity between the chromosomes are intermediate between two extremes. On one hand we have hybrids in which the affinity between the parental chromosomes is so slight that they will *never* conjugate, as, for instance, in the hybrids *Nicotiana Bigelovii* × *suaveolens* and *Bigelovii* × *glutinosa* (Goodspeed and Clausen, 1927) and *Raphanus* × *Brassica* (Kar-

pechenko, 1924). The other extreme is represented by hybrids in which the affinity between the parental chromosomes is so strong that they *always* pair. This condition can be illustrated by the hybrids *Geum rivale* \times *urbanum*, both with $n=21$ chromosomes, and *Tragopogon pratensis* \times *porrifolius*, both $n=6$ (Winge, 1926, 1928), and by varietal hybrids. Very similar to this is the case where the two parents have different chromosome numbers but *all* chromosomes from the parent that has the lowest chromosome number, pair with their homologues from the other parents, as in *Nicotiana paniculata* \times *rustica* (Goodspeed, Clausen and Chipman, 1926); and in all clear cases following the *Drosera* scheme. Between these two extremes are the cases described in the three *Crepis* hybrids. Apparently a large gradation exists between these extremes as manifested by the different percentages of failures to pair in different hybrids. Although in the *Crepis* case the degree of sterility to some extent seems to follow the degree of cytological irregularities and the degree of failure to conjugate, it does not always hold true, as shown by the *Tragopogon* hybrid which was almost sterile notwithstanding complete pairing; and similarly in *Lamium* hybrids described by C. A. Jørgensen (1927). The sterility might be caused mainly by non-balanced interaction of genes and perhaps has not much connection with the degree of mutual affinity between chromosomes. Only so far as mutual affinity between homologous chromosomes is *one* of the factors responsible for keeping up an *already established* balance between the genes constituting a certain genotype, can it be said that affinity between chromosomes has any bearing upon the question of sterility.

The present paper in all essentials was completed May 15, 1928, but was delayed five months by overwhelming experimental work. In the meantime two papers bearing on similar phenomena were published, namely, Maeda's paper on *Lathyrus* (1928) and Belling's paper on *Lilium* (1928*b*). Maeda also has applied the combination of Carnoy's and Navashin's fixatives for bringing out very conspicuously the spiral structure of the chromosomes. Belling's description of the chiasmata in *Lilium* (pp. 467-468) corresponds with the condition in *Crepis*, but the figures, as well as the earlier figures of *Utricularia* and *Hyacinthus*, apparently do not show such fine details as *Crepis* shows. Whether this is due to a profound difference in structure of the chromosomes or to the squeezing applied in Belling's investigations cannot be told.

TAXONOMIC RELATIONS OF THE FIVE SPECIES

According to a tentative taxonomic grouping (Babeock and Lesley, 1926, slightly modified) the five species discussed here would be classified as follows:

SUBGENUS A. Achenes beaked

Sec. III. **Barkhausia***Crepis bursifolia**Crepis taraxacifolia*Sec. IV. **Nemauchen***Crepis aspera**Crepis aculeata*

SUBGENUS B. Achenes not beaked

Sec. VI. **Eucrepis***Crepis tectorum*

The above sections are distinguished primarily by the form of the achenes. In *Barkhausia*, as tentatively used here, the achenes are all similar and definitely beaked. In *Nemauchen* they are of two shapes, the marginal achenes being unbeaked and the inner ones beaked. In *Eucrepis* the achenes are all unbeaked (or in a few species very shortly beaked). The achenes of all but one of these five species have been illustrated (Babeock and Lesley, 1926, fig. 3, *g*, *h*, *h'*; fig. 5, *r*). In *Crepis aculeata* the achenes resemble those of *C. aspera* but are larger, while the marginal ones are less prominently angled and the inner ones proportionately shorter beaked.

Considering the meiotic behavior and fertility of the hybrids herein discussed with reference to classification of the species involved, it is clearly shown in tables 1 and 2 that the *Crepis aspera* \times *aculeata* hybrid displays the lowest amount of chromosome irregularities in meiosis I with a large amount of regular pairing and very few pollen mother cells showing detached chromosomes. The percentage of irregularities in meiosis II is also low. In the *C. taraxacifolia* \times *tectorum* F_1 , on the other hand, less than one-third of the pollen mother cells examined show regular pairing and a very large percentage have detached chromosomes. The *C. aspera* \times *bursifolia* F_1 showed even less regularity in pairing in its pollen mother cells, but the number of detached chromosomes observed was not so high as in

the *taraxacifolia* × *tectorum* hybrid, while the percentage of irregularities in meiosis II was nearly as large. In other words, the last two hybrids, one involving different subgenera and the other, different sections of the same subgenus, display much greater irregularity during meiosis in their pollen mother cells than does the hybrid between two species of the same section.

Data on the proportion of apparently good pollen grains formed are in close agreement with the foregoing. Pollen counts of 500 to 1000 grains per plant were made in most cases. The pollen was stained with acetocarmine and showed great variation in size and staining capacity of the grains. Only those grains of average or large size and deeply stained were counted as "good." In the case of *C. aspera* × *aculeata* three plants gave 36, 40, and 48 per cent respectively of good pollen. Of the *taraxacifolia* × *tectorum* hybrids three plants had from 1 to 2 per cent of good grains. Only one plant of *aspera* × *bursifolia* was examined and it gave less than 1 per cent of good pollen. Fortunately, however, pollen counts were made on three plants of *bursifolia* × *aculeata* and two plants of *taraxacifolia* × *aspera* and all these gave only 1 per cent or less of good pollen.

The proportion of viable egg cells produced in *Crepis* hybrids is usually larger than the proportion of viable pollen formed. The only data bearing on this in the three hybrids discussed above are Haney's observations on the amount of open-pollinated seed produced, and under conditions at Berkeley this is rather variable. The observations made, however, indicate general agreement between amount of open-pollinated seed produced and proportion of good pollen grains present.

The data on meiosis, pollen formation, and fertility may be summarized as follows:

Sub-genus	Section	Hybrids	Meiotic irregularities observed	Percentage of "good" pollen formed	Percentage of seeds set under open-pollination
A	IV	<i>aspera</i> × <i>aculeata</i>	Rather few	35-50	30-40
A	III	<i>taraxacifolia</i> × <i>tectorum</i>			
B	VI	<i>tectorum</i> × <i>aspera</i>	Very numerous	1-2	Few or none
A	IV	<i>aspera</i> × <i>bursifolia</i>			
A	III	<i>bursifolia</i>	Very numerous	1±	3-4

These results are in fairly good agreement with the taxonomic relationship as determined by comparative morphology. It will be noted, however, that meiotic irregularities are just about as high and fertility nearly as low in *aspera* \times *bursifolia* (both in Subgenus *A*) as in *taraxacifolia* \times *tectorum* (Subgenus *A* \times *B*). This must indicate profound physiological diversity between *Barkhausia* and *Nemauchenes*. It provides additional reason for maintaining these species of *Nemauchenes* as a separate group from their close relatives in *Barkhausia*. It also shows, however, that when the relationship between two species is below a certain threshold value, the meiotic irregularities and degree of sterility exhibited by hybrids between them are of little value in determining the degree of relationship between the species. These criteria, in other words, are useful only within certain limits in the study of taxonomy, and should be considered only in relation to other criteria such as number and morphology of the chromosomes, and the distribution, ecology, and comparative morphology of the plants themselves.

SUMMARY

1. This paper deals with the two pure species, *Crepis aspera* L., and *C. bursifolia* L., together with the following three hybrids: *Crepis aspera* \times *C. bursifolia*, *C. taraxacifolia* Thuill. \times *C. tectorum* L., and *C. aspera* \times *C. aculeata* (DC.) Boiss.

2. All five species have $n=4$ chromosomes. After a discussion of methods of fixation and staining, meiosis in the two species, *C. aspera* and *C. bursifolia*, is described. In *C. bursifolia* one chromosome pair, which is shorter than the other three pairs, can be recognized through all phases. The chromonema thread in the early zygo-phase shows chromomeres, and parasynapsis has been found. In the diplophase the chromonema coils up into a spiral filament which in the diaphase or diakinesis appears to be doubled by splitting. In this phase chiasmata between the partners of the bivalent chromosomes are often seen. These chiasmata may be regarded as the result of interchange accomplished at an earlier stage, before the chromosomes have become shortened by the spiral coiling of the chromonemata. Extraction of the crossed-over chromatids would occur in the heterotypic anaphase.

3. In the hybrid, *C. aspera* \times *bursifolia*, the structure of the chromosomes can be recognized as similar to that of the parent species and chiasmata can be found between the partners of the bivalent chromosomes. In pollen mother cells where four pairs of chromosomes are present, a pair consisting of two unequal partners is seen and the short *bursifolia* chromosome can be followed through and recognized in all phases.

4. Of the three hybrids, *C. taraxacifolia* \times *tectorum* and *C. aspera* \times *bursifolia* show comparatively many irregularities during meiosis, while *C. aspera* \times *aculeata* is fairly regular (table 1 and 2, p. 412).

5. The data on meiotic irregularities in these hybrids are in close agreement with the taxonomic relations between the parental species; also the data on percentage of "good" pollen formed in the hybrids, and their fertility, agree well, in the main, with the irregularities observed in meiosis as well as with the taxonomic relations, with the exception that, if their relationship is below a certain threshold value, these criteria cannot be used for determining how remote two species are from each other.

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EXPLANATION OF PLATES

For slides of sectioned material Leitz' 2 mm.-apochromatic objective, aperture 1.4, together with binocular eye-piece $\times 15$ has been applied; for iron-acetocarmine smears, Leitz' 3 mm.-apochromatic objective, together with the same eye-piece. Parallel rays from a small circular source of light of great intensity were sent through Leitz' achromatic condenser aperture 1.4 for illumination.

The magnification of sectioned material is about $\times 2100$. In the preparations treated with acetocarmine the swelling of the chromosomes caused by the acetic acid just compensates the minor magnification of the 3 mm.-objective as compared with the 2 mm.-objective, so that all pollen mother cells reproduced appear of practically the same average size.

In the tetrads and polyads stress has been laid mainly upon number, size, and shape of nuclei and cells, and the structure shown in the nuclei of these must be regarded only as a kind of signature.

PLATE 58

Crepis aspera.

Fixation: Carnoy-Navashin. Sectioned material. Stained in gentian violet-iodine-orange G.

Fig. 1. Zygothase; the thick chromomeres probably have been formed by parallel union of pairs of chromomeres.

Fig. 2. *a*. The chromatic thread opens up, showing its doubleness; *b*, one of the four bivalents in diplophase, a little later. Seven twists of its partners around each other were counted; note the chromomeres.

Fig. 3. The four bivalents in one pollen mother cell in diplophase; the partners still much twisted around each other.

Fig. 4. Later diplophase; four bivalents are seen; the partners untwist but the chromatic thread coils up in a spiral.

Figs. 5-9. Diaphases (diakinesis). In all pollen mother cells 4 bivalents can be counted. Note the two spiral chromatids in each chromosome in this phase. Chiasmata are clearly seen in figures 5-8; in figure 6 one bivalent with a chiasma is drawn outside the nucleus in order to show the underlying bivalent; in figure 7 one bivalent probably has two chiasmata; in figure 8 there is one clear chiasma, the two partners forming a cross. Figure 9 shows different shapes of bivalents in one pollen mother cell.

Figs. 10-11. Two heterotypic metaphases; different shapes of bivalents.

Crepis bursifolia.

Sectioned material; fixed and stained as the preceding ones.

Figs. 12-13. Diaphases of two pollen mother cells; different shapes of bivalents (rings, crosses). Note the short pair and the spiral chromatids.

Figs. 14-15. Two heterotypic metaphases, each with 4 bivalents; in figure 14 three crosses and perhaps one rod; in figure 15 a double J, two rings and one rod. Note the short bivalent.

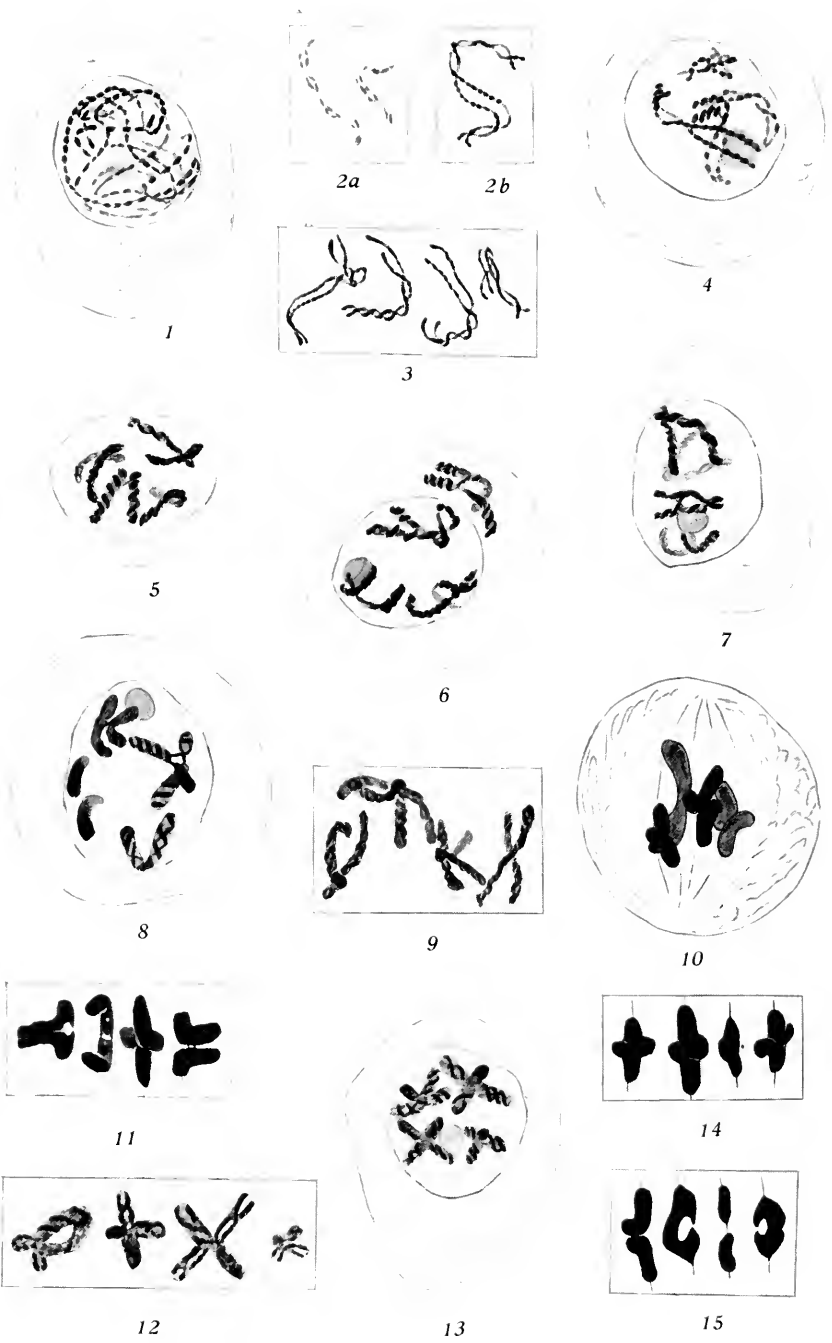




PLATE 59

Crepis bursifolia.

Fig. 16, heterotypic anaphase; fig. 17, homotypic metaphase; fig. 18, homotypic anaphase; note the short chromosome in all four groups of chromosomes.

Crepis aspera × *bursifolia*, F₁.

Sectioned material. Fixation: Figure 30, Carnoy; all the others Carnoy-Navashin and all stained in gentian violet-iodine-orange G.

Figs. 19-22. Selected diaphases in which four bivalents occur. Note the conjugation of the unequal pair and the chiasmas in figures 19-21; in figure 20 it has been necessary to draw one bivalent outside the nucleus.

Figs. 23-29. Heterotypic metaphases. Figure 23 has 4_{II}, figures 24-25 3_{II} + 2_I, figure 26 has 2_{II} + 4_I; in figure 27 there is 1_{II} + 6_I and in figure 28, finally, all 8 chromosomes are unpaired (non-reduction). In figure 23 probably the left bivalent is the unequal one; in figure 24 the two unpaired chromosomes are the unequal ones, in figure 25 these are conjugated, resembling a pair of XY chromosomes, while two equal chromosomes here are unpaired. In figure 29 (not complete) an unequal pair very clearly shows.

Fig. 30. Heterotypic anaphase giving 4 long chromosomes in one of the daughter nuclei and 3 long and one short in the other.



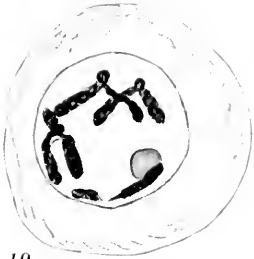
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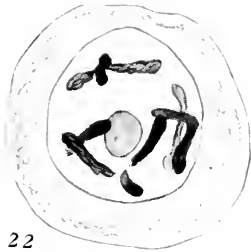
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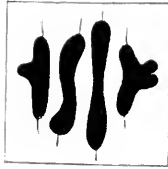
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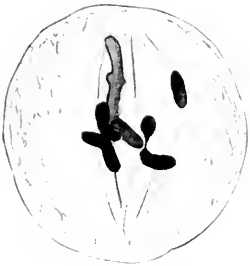
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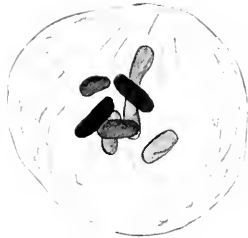
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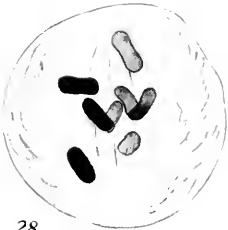
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PLATE 60

Crepis aspera × *bursifolia*, F₁.

Figures 31-34 from sectioned material, figure 31 from Carnoy fixation, the others from Carnoy-Navashin, all stained in gentian violet-iodine-orange G. Figures 35-41 are from iron-acetocarmine smears, previously fixed in Carnoy and preserved in 70 per cent alcohol.

Fig. 31. Heterotypic anaphase; one chromosome eliminated, the short chromosome splitting in the equatorial plane.

Figs. 32, 33. Homotypic anaphase. Figure 32 has five chromosomes at one pole, two at the opposite pole, and one (the small one?) splitting outside both daughter groups of chromosomes.

Fig. 34. Homotypic anaphase in one section; the left picture represents the upper cap of the pollen mother cell. The short chromosome is seen in this cap.

Figs. 35-41. Polyads representing the tetrad stage. Figure 35 apparently will form a diad with two and four nuclei respectively. Figure 36 probably represents a diad, but possibly it might belong to interphase (interkinesis); three micronuclei are seen. Figures 37 and 39 are tetrads with micronuclei. Figure 38 is a pentad, figure 40 a tetrad, but one of its cells is very small and one is very large with three micronuclei. Figure 41 is a true triad.

Crepis taraxacifolia × *tectorum*, F₁.

Smear preparations fixed previously in Carnoy and stained in iron-acetocarmine.

Figs. 42-45. Heterotypic metaphases. In figure 42, 4_{II}; in figure 43, 3_{II} + 2_I. Figures 44 and 45 have 2_{II} + 4_I each, but in figure 44 the bivalents form a cross and a rod, respectively, while in figure 45 they form two crosses.

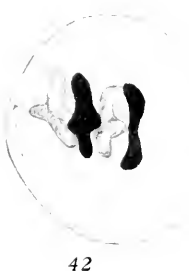
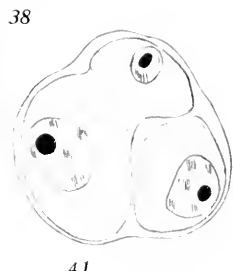
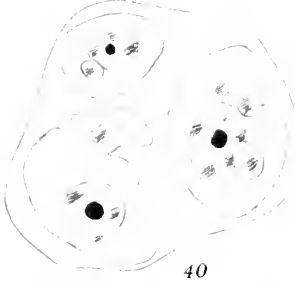
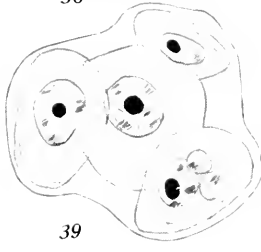
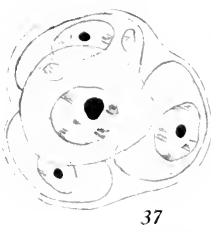


PLATE 61

Crepis taraxacifolia × *tectorum*, F₁.

Fig. 46. Homotypic anaphase with four chromosomes in all four groups.

Fig. 47. Homotypic anaphase with 3/2/3 chromosomes in the upper of the two division figures and 4/1/3 in the lower one.

Fig. 48. Represents the tetrad that probably would result from the division shown in figure 47, the figures in the nuclei giving the number of chromosomes in the four nuclei and three micronuclei.

Fig. 49. A nonad of which three nuclei probably have three chromosomes each, one probably has two chromosomes, and five are micronuclei with probably only one chromosome each, giving the total of sixteen chromosomes in the nine nuclei.

Figs. 50-51. Tetrads with micronuclei; in figure 51 one of the cells is very small.

Fig. 52. A true diad.

Figs. 53 and 54. Triads; in figure 54 one of the cells has two nuclei.

Crepis aspera × *aculeata* F₁.

Iron-acetocarmine smears. The material previously fixed in Carnoy.

Figs. 55-56. Heterotypic metaphase; in figure 55 4_{II} (the most common case), in figure 56 3_{II} + 2_I.

Fig. 57. Heterotypic anaphase; one chromosome lagging and splitting.

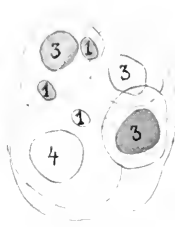
Fig. 58. Tetrad; one of the few cases where micronuclei were found.



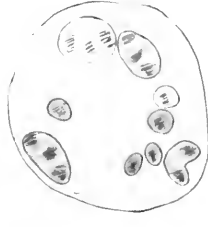
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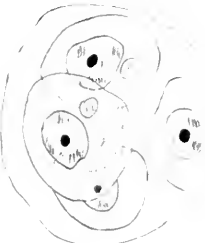
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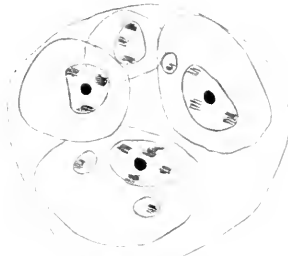
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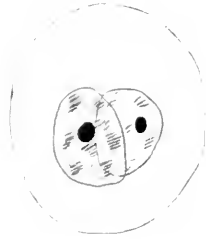
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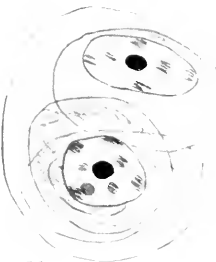
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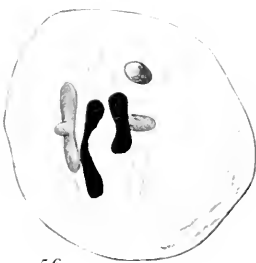
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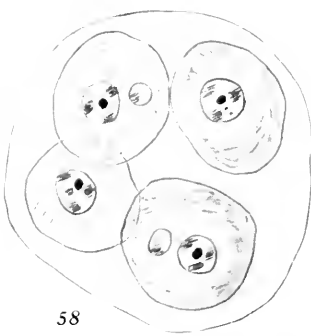
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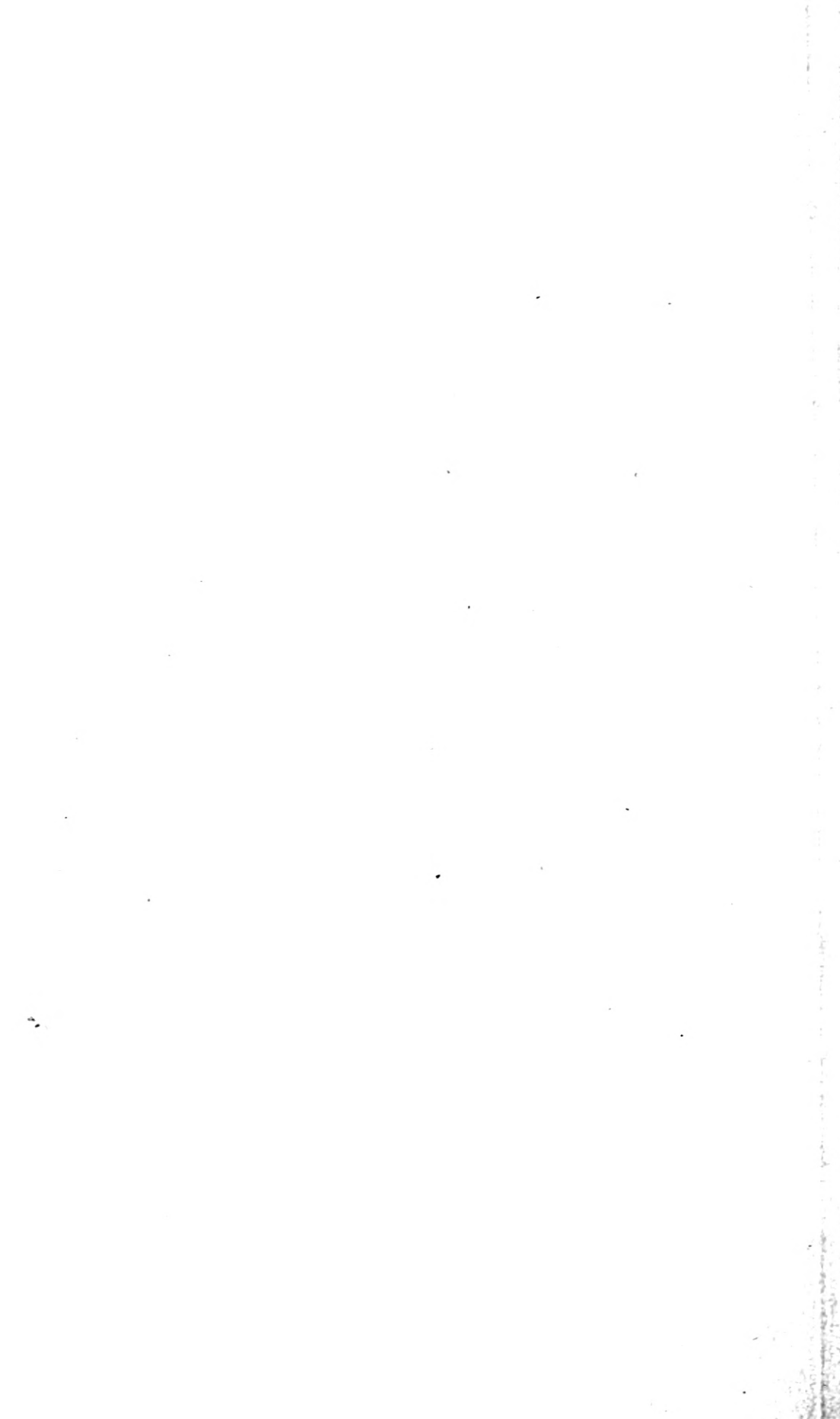
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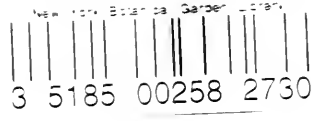
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