UNIVERSITY OF CALIFORNIA PUBLICATIONS
IN
AGRICULTURAL SCIENCES

Vol. 2, No. 5, pp. 191-204, plates 36-28 October 19, 1920

INTERSPECIFIC HYBRIDS IN CREPIS

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

BY

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Although the problem of the mechanism of heredity may be said to have been solved by Morgan and others[1] 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 writers[3] 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	*capillaris*×*tectorum*	6 *tectorum* 5 *capillaris* 1 intermediate	2	All failed to pass cotyledon stage	12
Z 5	*capillaris*×*tectorum*	All *tectorum*, showed hybrid vigor	2	All failed to pass cotyledon stage	6
Z 7	*capillaris*×*tectorum*	All *tectorum*, showed hybrid vigor	2	All failed to pass cotyledon stage	3
Z 8	*capillaris*×*tectorum*	Intermediate	2	All failed to pass cotyledon stage	1
Z 10	*tectorum*×*capillaris*	All *tectorum*, showed hybrid vigor	1	All failed to pass cotyledon stage	12
Z 12	*capillaris*×*tectorum*	Small and distorted, abnormal	1	Failed to pass cotyledon stage	1
Z 13	*capillaris*×*tectorum*	Small and distorted, abnormal	2	All failed to pass cotyledon stage	5

Z 3 F_1 (18.42 P_{21} × p2 P_{51}) 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 F_1 (18.58 P_4 × p2 P_{10}) 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 F_1 (18.204 P_6 × p2 P_{51}) CAPILLARIS × TECTORUM

Eight F_1 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 F_1 (18.201 P_1 × p2 P_{51}) 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 F_1 (p2 P_{51} × e4 P_2) 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 F_1 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 F_1 (18.42 P_1 × p2 P_{51}) 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 F_1 (e14 P_7 × 212 P_{21}) 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 apeared 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. Metz[5] shows a diagrammatic gradation of chromosome numbers for different species of Drosophila. Hance[6] applies the same idea to the origin of chromosome number variations in Oenothera species. Rosenberg[7] 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. Bridges[8] 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 *Œnothera 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. × 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* ♀ × *C. capillaris* ♂ (above). F_1 hybrids, *C. capillaris* ♀ × *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
c
c
4
5

PLATE 37

Teratological tissue of F_1 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.


b
c
a

PLATE 38

Fig. 1. Teratological tissue in F_1 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 F_1 hybrid *C. capillaris* × *C. tectorum* (Z 5) showing two J's, one V, and rodlike ones. × 1500.

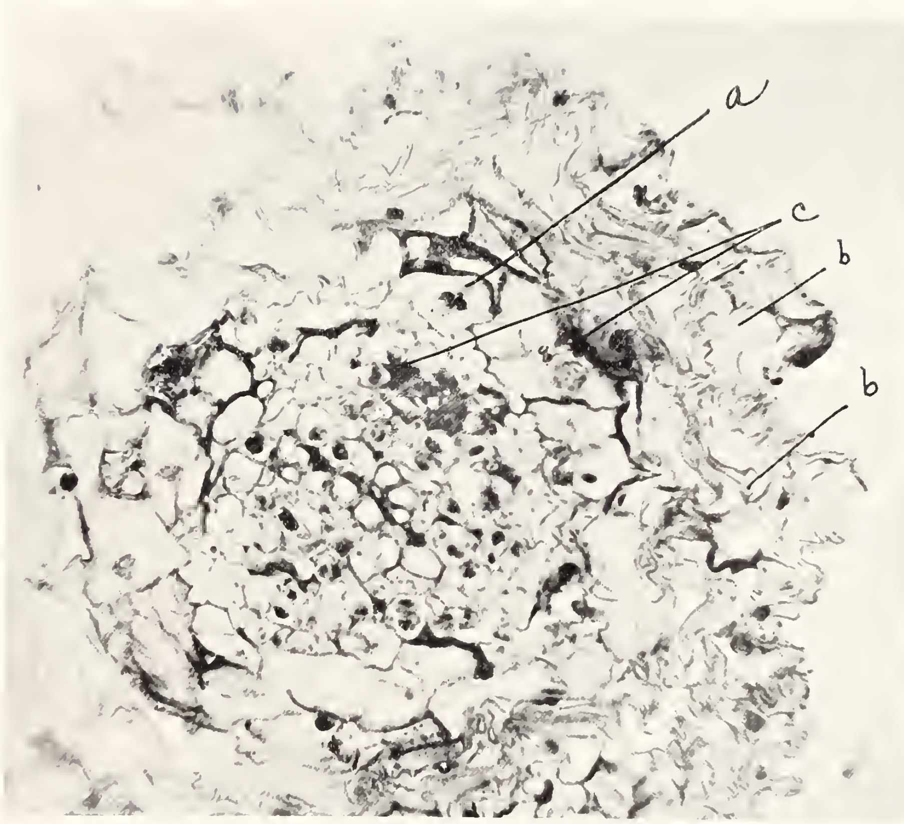

Fig. 1

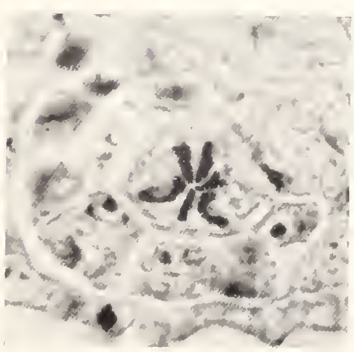

Fig. 2

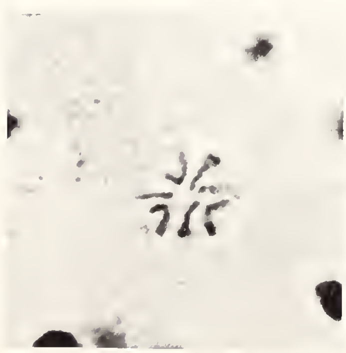

Fig. 3