CYTOLOGICAL AND MORPHOLOGICAL INVESTIGATIONS ON GYNODIMORPHIC AND NORMAL FORMS OF *RANUNCULUS ACRIS* L.

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INTRODUCTION

The present studies were undertaken to determine whether certain morphological deviations from the typical vegetative and floral characteristics of *Ranunculus acris* L. are accompanied by variations from type in the nucleus. Work has been in progress for a number of years, and has involved many races of *Ranunculus acris* L., collected in Russia, Czechoslovakia and America, and cultured at CHARLES' UNIVERSITY, Prague, COLUMBIA UNIVERSITY and the UNIVERSITY of MINNESOTA.

Specifically, the investigation has involved:

(a) A study of the superficial morphological features of aberrant individuals and races of *R. acris* and the assembling of materials for genetic and cytological investigation.

(b) A cytological investigation of the behavior of the nuclei during somatic and reduction divisions with special reference to the problem of the individuality of the chromosomes.

(c) A study of the possible correlation between chromosome number and chromosome formulae with superficial morphological characteristics in aberrant races.

It is a pleasure to acknowledge my indebtedness for many valuable suggestions to Doctor B. NEMEC under whose direction this problem was begun. I wish also to express my obligations to Doctor R. A. HARPER and Doctor C. E. ALLEN for helpful criticism, to academician Doctor S. G. NAWASCHIN for the preparations of satellites, to Doctor C. O. ROSENDAHL in whose laboratories the work was completed for advice and criticism, and to Doctor J. ARTHUR HARRIS for help in the preparation of this paper.

EARLIER OBSERVATIONS ON GYNODIMORPHISM IN *Ranunculus acris* L.

Gynodimorphism has been described several times in different representatives of the family Ranunculaceae. Plants of *R. acris* L. with small flowers and abortive anthers were first reported growing with normal plants on the meadows at Politz, Mettau, by VELONOVSKY (1900). He says:

> "Es sitzen überall auf den Blütenasten auffallend kleine Blüten (2–3 Mal kleiner als bei der Normalpflanze) welche kleine, mehr keilformige Petalen besitzen und auf dem Blütenboden nur Fruchtknoten aber keine Antheren tragen. Diese sind ganz unansehnlich unter den Fruchtknoten als Höcker versteckt. Die Fruchtknoten gelangen regelmässig zur vollstandigen Entwicklung und reifen sogar zu Früchten."

MAGNUS (1900), discussing the phenomenon described by VELONOVSKY, pointed out the importance of the gynodimorphic race:

> "Auch die Bestaubungseinrichtungen der einzelnen Arten sind nichts starres, sondern etwas Gewordenes und Werdendes."

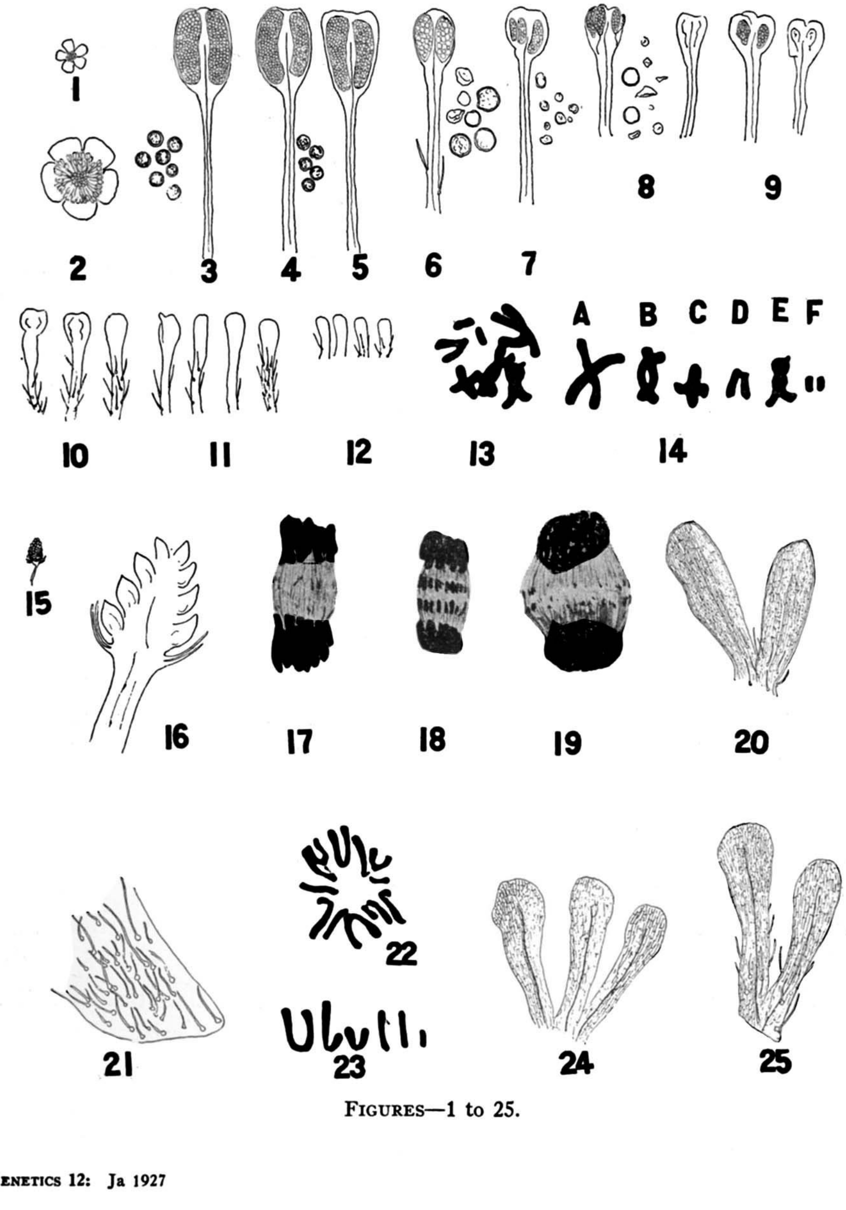
Near Grünberg, Germany, SCHMIDT (1911) found *R. acris* L. with small flowers, from ½ to ¼ of the size of the normal, with anthers fully aborted, petals and stamens often modified, increased in number, and even sometimes forming absolutely double flowers.

TURRILL (1919) mentioned the occurrence of similar flowers in *R. auricomus*, *R. acris* and *R. bulbosus*:

> "Their stamens were very much reduced in size and produced little or no pollen. Similar unisexual states of both *Ranunculus bulbosus* and *R. acris* have been found on the lawns at Kew."

Gynodimorphic flowers of *Ranunculus polyanthemus* were observed by DOMIN (1923). Similar flowers in other Ranunculaceae are mentioned by PENZIG (1922).

The somewhat frequent occurrence of these aberrant types in Ranunculus and its relatives suggested the desirability of a cytological investigation with a view to determining whether nuclear, and particularly chromosomatic, phenomena are associated with the externally visible morphological characteristics.



FIGURES—1 to 25.

MATERIALS OF PRESENT INVESTIGATION

Gynodimorphic plants of *Ranunculus acris* L. were found by the writer at Czarskoe Selo, near Petrograd, Russia, in practically every place where one would expect to find the normal plants of *Ranunculus acris*. The pure gynodimorphic plants were rather rare as compared with the normal plants. Intermediate stages between the normal plants and the gynodimorphic were more abundant.

In figure 1 a typical gynodimorphic flower is compared with the normal (figure 2). The flowers of the gynodimorphic plant were small, their petals averaging from 3 to 4 mm in length, as compared with petals 10–11 mm in length in the normal plants. The stamens were abortive and were reduced to small staminodia. The pigment of the petals in the gynodimorphic flowers was very pale, as contrasted with the bright yellow pigment of the normal plants. Such vegetative characters as shape and size of leaves, the distribution of the hairs, and other characters were also conspicuously affected and involved in the variations. Intermediate forms, which in the degree of reduction of the anthers, and in size of the flowers approached either the extreme gynodimorphic or the normal form, were present.

The range of variations of stamens and pollen grains which have been observed in these forms is represented in figures 3 to 12. An anther and pollen grains of a normal *R. acris* are illustrated in figure 3. Figures 4 and 5 represent forms with slightly modified anthers and with apparently normal pollen. Forms in which the filaments are long, but the pollen sacs are rather strongly modified and provided with pollen grains irregular in size and shape are shown in figures 6 and 7. In the forms represented in figures 8, and 9 the anthers are further reduced and approach more nearly to the true staminodia. The pollen grains, if present, are extremely irregular. A form in which the pollen sacs, though present, are empty is shown in figure 9, (right). Some typical staminodia are shown in figures 11. The last stage of the reduction of the anthers to very small staminodia of a typical gynodimorphic plant is represented in figure 12.

A comparison of the figures of the forms discussed shows that there probably is a correlation between the degree of reduction of the anthers and the development of the hairs on the filaments of the stamens. The size and shape of the pollen grains in the intermediate forms are highly variable. The form shown in figure 6 has a great number of very large and irregular pollen grains. The form shown in figure 7 has on the contrary a great proportion of small-sized irregular pollen grains. In the form shown in figure 8 there are still more irregularities in the shape and size of the pollen and a still greater proportion of shriveled grains.

In the spring of 1922 the writer collected gynodimorphic and normal plants of *R. acris* from the environs of Petrograd, Russia. In the fall of 1922 the seed material from these two forms was sown in the botanical garden of CHARLES' UNIVERSITY, Prague. In the summer months of 1923 the cultures flowered abundantly. The progeny of the gynodimorphic plants showed very pronounced variations of the vegetative characters and of the floral structures. These forms have received especial attention from the cytological standpoint.

MITOSES AND MEIOSES IN THE NORMAL AND GYNODIMORPHIC *R. acris*.

In the somatic mitoses (SOROKIN 1924) the normal plants were found to have 12 chromosomes, whereas the gynodimorphic had 18. Gynodimorphic plants with other chromosome numbers were also found later In both forms satellites were present in two chromosomes. In the normal plant one small satellite was connected with one chromosome, while the other chromosome had a satellite of slightly larger size. In the gynodimorphic plant three satellites were present, two of them connected with one chromosome, and one with another chromosome. The behavior of the nucleolus, which underwent regular divisions before the division of the nucleus in both normal and gynodimorphic forms, has been discussed (SOROKIN 1924).

The meiotic phase of one of the normal races of *R. acris* is described in detail in a separate paper, SOROKIN (1926). Here an inquiry into the individuality and morphology of the chromosomes will be considered.

In the cytological work several fixing reagents were used. For different purposes the solutions proved to be of unequal value. For root tips, the best preparations were obtained from the material fixed in chrom-formalin according to the new formula given by Professor B. NEMEC, which he recommends for fixing mitochondria,[1] and which was excellent for the chromosomes also. For studies of the reduction divisions, the best material was obtained from Flemming's medium. While Flemming's strong solution produced very pronounced plasmolysis, fairly good results were obtained from the picric-acetic-sulphuric acid killing solution. It is interesting to point out that the results of the fixation of the same plant with the same solution varied greatly and seemed to depend upon the

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[1] 100 cm^3 of 1 percent CrO_3 and 8 cm^3 of formaldehyde were allowed to act on the material for 6–8 hours, after which the material was subjected to the action of a new prepared portion of the same solution for 16–18 hours.

time of day and upon the temperature of the air. The best results were always obtained from the material fixed during warm weather in the morning. For the study of the somatic mitoses sections were cut 7.5μ thick, and for the reduction divisions from 10μ to 15μ, and were stained with Heidenhain's iron alum haematoxylin.

The figures of the accompanying illustrations are all drawn with camera lucida to corresponding scales. Namely: mitoses and meioses—Zeiss Apochr. 3 mm Ap. 1.30, comp. oc. 12; anthers, staminodia and hairs on the calyx of the progeny of a gynodimorphic plant crossed with normal *R. acris*—Leitz, 3.3; pollen grains 3.7, all these figures are diminished four times. Flowers—natural size. Range of variations of stamen and pollen grains in forms found growing wild—enlarged about 30 times.

EARLIER WORKS ON THE INDIVIDUALITY OF THE CHROMOSOMES

Studies on the individuality of the chromosomes have recently acquired great importance. The relative size of the chromosomes, peculiar shape, and especially the presence of satellites (S. NAWASCHIN, 1912) and constrictions (SAKAMURA 1915, 1920) furnish criteria for distinguishing the members of a given complement, or using the terminology of S. NAWASCHIN, they characterize the *idiogram* or the specific arrangement of the diploid nuclear plates.

S. NAWASCHIN (1912) discovered the satellites of the chromosomes in *Galtonia candicans*. The following data are given by him (1912, 1915) concerning the nature of these peculiar structures. The satellites are much smaller than the chromosomes. In the metaphase of the somatic mitoses they are attached by means of delicate threads to a certain chromosome pair. They appear already during the prophases, where they were observed on the surface of the nucleoli. During the maturation divisions the satellites fuse with their chromosomes, reappearing again in the pollen grains during the divisions of the primary nucleus.

The writer was very fortunate to receive from Prof. S. G. NAWASCHIN excellent preparations of *Galtonia candicans* showing satellites of the somatic chromosomes in prophases and metaphases. Slides made from the material killed with sublimate osmic and stained with Delafield and eosin, showed in the prophases the dark purple stained satellites situated on the opposite sides of the nucleolus, which was stained pink with this combination of stains.

In the diploid nuclei of *Drimiopsis maculata* BARANOV (1926) found four large and 12–16 small satellites. From the beginning of the prophases till the moment of the formation of the chromosomes, the satellites are found on the surface of the nucleoli. Later on they are picked up from this position by the delicate threads extending from the ends of the chromosomes, and appear to be connected with the chromosomes during the metaphases and anaphases. In the heterotypic division, the satellites were observed only in leptonema and synizisis stages. In the same paper BARANOV refers to the slides found now at the TIMIRIAZEFF'S INSTITUT, Moskaw and to the paper of S. G. NAWASCHIN given in Vienna in 1913, in which the arrangement of the satellites on the nucleoli in the prophases is clearly demonstrated.

In a recent paper M. NAWASCHIN (1925b) describes the satellites in diploid and triploid *Crepis capillaris*, where they are found on the surface of the nucleoli during the prophases. Two satellites are found on the nucleoli of the diploid race, and three satellites are recorded on the nucleoli of the triploid individuals.

The number of plants in which the satellites are found is steadily increasing. The important literature on the subject is outlined in the book by TISCHLER (1921–1922), and in the articles by TAYLOR (1924, 1925a, 1925b, 1925c), NAWASCHIN (1925a) BARANOV (1926). In addition to the recorded literature a recent paper by MEYER (1925) should be mentioned. He confirmed the previous observation of the satellites in somatic mitoses of *Leontodon autumnaeis*. The satellites were found also in the polyploid nuclei of the tapetal cells, but absent in the chromosomes during the reduction division. NAWASCHIN (1925a) published the first results of his intensive studies on the chromosomes of fourteen species of Crepis. In all the investigated forms two distinct types of the chromosomes were found. The first type was represented by the two-armed, slightly constricted chromosomes, the arms being usually of unequal length. The second type included rod-shaped chromosomes with small proximal satellites. Miss EMME (1925) studied the idiograms of a great number of varieties of wheat. She found that endemical forms from Asia mostly do not have satellites. The endemical forms from Africa have one satellite, and belong to the asymmetrical race. The wheat varieties which do not belong to any of the endemical groups have different karyotypes, partly symmetrical with no, or with two satellites, and partly asymmetrical with one satellite. In spite of the fact that more than one hundred plants with satellites are recorded, their nature is often misunderstood and confused with some of the other typical characters of the chromosomes. The conception of the satellite of DARLINGTON (1926) as being "the result of a local discontinuity which occurs in different chromosomes at different positions—bisecting the chromosome or cutting off a minute element, which will form, in the contracted state, a satellite" is entirely wrong. The satellites have a different origin and always represent hereditary morphological characteristics of the chromosomes. As to the phenomenon observed by DARLINGTON, it must have been the type of temporary constrictions which sometimes occur in the chromosomes.

TAYLOR (1925c, 1926) recognized three notable features of the chromosomes, namely constrictions at the zone of fiber attachment, constrictions unrelated to the fiber attachment, and satellites. In regard to the second type of morphological characters of the chromosomes TAYLOR (1926) thinks that there does not seem to be distinct demarcations between this kind of phenomena and the original satellites. Somewhat similar conclusions could be made from the paper of DELAUNAY (1925) on Ornithogalum. In different species of this plant he found satellites of unequal length. The longest satellites being in *O. umbellatum* and *O. Narbonense,* the shortest in *O. oligophyllum.* This conception of the satellites differs from the original description of NAWASCHIN and his pupils. In order to avoid misunderstanding it would be desirable to restrict the word "satellite" to the phenomena which approach the original satellites in *Galtonia candicans.*

Besides the two permanent types of constrictions, one related to the fiber attachment, the other independent, there are sometimes found in the chromosomes temporary constrictions, which are not distinct hereditary characters, and which may appear and disappear at certain periods of the development of the chromosomes. The constrictions of the chromosomes in prophases of the somatic and heterotypic divisions, and especially the constrictions related to the interchange of the segments of a certain pair of the chromosomes belong to this temporary type. These constrictions occur in *Ranunculus acris* (SOROKIN 1926) shortly before diakinesis. The constrictions of the chromosomes with the subsequent fracture (BELLING 1925) should also be considered in the class of temporary constrictions.

THE INDIVIDUALITY OF THE CHROMOSOMES IN *R. acris*

In *R. acris* the individuality of the chromosomes is readily demonstrable. The somatic chromosomes differ in size, shape, presence of constrictions, and satellites.

The formula for the somatic chromosomes of the normal race of *R. acris* from Czarskoe Selo, Petrograd, is $2A+2B+2c+2d''+2e+2f=12$ chromosomes. The chromosomes in this formula are arranged first of all according to differences in size. The two A-chromosomes are very large.

They are, therefore, indicated by capital letters. The B-chromosomes are medium large, while the c-, d- and e- chromosomes are medium and the f-chromosomes are very small. The second distinguishing character is shape. The A-chromosomes are V-shaped, with both arms long, of equal size and with median fiber-attachment. These chromosomes are easily recognized in different stages of mitosis, even under unfavorable conditions. The B-chromosomes are hook-shaped with one very long arm, which is sometimes even a little longer than the arms of the A-chromosomes, and with another arm one-third as long as the first. The fiber attachment is subterminal. A very marked constriction is found between the two arms of the B-chromosomes. The c-chromosomes are of regular small v-shape. The d-chromosomes are hook-shaped, with one arm one-fourth as long as the other. Either both of the d-chromosomes are provided with the satellites and constitute the symmetric race, according to the terminology of S. NAWASCHIN (1915) or only one of the d-chromosomes has a satellite and can form an asymmetric race. Accordingly the presence of one or two apostrophics in the formula after the letter d will indicate the condition. The e-chromosomes are either of rod or hook shape and without any particular properties in the somatic mitoses. The f-chromosomes are very small rods, and are always found to be in the center of the metaphasic figures.

The shape and size of the individual chromosomes in the meiotic phase of the normal *R. acris* from Czarskoe Selo are represented in figures 13, 14. The A-chromosomes form a large cross in diakinesis (figure 14A). The B-chromosomes (figure 14B) form two curves which are crossed once or twice, the c-chromosomes form a small cross (figure 14C) the d-chromosomes are connected on one side (figure 14D), the e-chromosomes, (figure 14E) remain for a very long time connected with the nucleolus, and the f-chromosomes (figure 14F) of very small size, form two very small rods.

In the gynodimorphic plants the conditions as to the individuality of the chromosomes are extremely complicated. The original gynodimorphic plant which is represented in figures 1 and 12 and which was used as a parent plant for the subsequent generations had 18 chromosomes. Because of limited material these chromosomes were not identified exactly as to their individuality, but it did appear that the chromosomes of this plant, though present in the number of 18 or in a trivalent number, did not represent a triploid race, because they could not be arranged in six trivalent sets. Chromosomes A and B could never be found in triple numbers. As will be shown later purely gynodimorphic plants with varying numbers of chromosomes have been found among the offspring of the original gynodimorphic plant with 18 chromosomes crossed with the normal plants. The figures in table 1 which gives the somatic formulae show that these numbers were 13, 15 and 18. It must be pointed out that the original gynodimorphic plant which was used in the experiment as the parent plant was extremely tall (about 120 cm) while the gynodimorphic plants with 13 and 15 chromosomes did not exceed 50 cm.

DESCRIPTION OF THE PROGENY OF A GYNODIMORPHIC PLANT CROSSED WITH NORMAL *R. acris*

The seeds from a gynodimorphic plant of *R. acris* grown isolated in the experiment garden of the AGRICULTURAL ACADEMY in Czarskoe Selo, near Petrograd, Russia, and fertilized by the pollen of normal plant of the same species, were sown in Prague, Czechoslovakia. The progeny was extremely variable in morphological characters. The size and shape and the degree of lobing of the leaves from entire to finely dissected, the distribution of the hairs on the leaves, varied considerably in all these forms. Gigantism and dwarfness were quite usual phenomena. Some plants were remarkably tall, others were of the type of the nanella mutations. But most conspicuous were variations in the floral structures. About one-quarter of the offspring were typical gynodimorphic. Most of the plants, however, showed intermediate stages between the purely gynodimorphic and the normal types. Only a very limited number of wholly normal plants could be found among the offspring.

Among other remarkable variations in the floral structures were reduction of the size of the sepals and petals and increase in the number of these organs. Some of the plants did not flower at all during the first season, so it was impossible to give accurate percentage of the different types of flowers.

The number of the somatic chromosomes of the different types of forms and the reduction division of the forms in which the anthers were not abortive were studied. The following description will illustrate the relations between the morphological characters and the cytological structures.

(a) *Mutations.* Among the progeny of the above plant, two forms were distinguished as mutations. They showed profound modifications of the structure of the flowers and were characterized as independent, absolutely different races, without any intermediate transition forms between them, and the other forms. They were called "Open buds" and "Asepalis." Both these plants began to flower in my cultures earlier than any of the others, and later on they produced abundant and apparently normal seeds.

The "Open bud" form showed complete reduction of sepals, reduction of petals to small scales, reduction of the anthers to staminodia and comparatively normal pistils, which were situated on a rather elongated receptacle. The whole aspect of the flower, of which figure 15 gives an illustration, was very unusual. The buds of this form were completely open and the young pistils were exposed from a very early stage. Longitudinal section of a bud, figure 16, gives an illustration of the parts of the flower of an "Open bud" mutation. The exact number and the individuality of the somatic chromosomes of this form has not been determined for want of material. There is, however, some evidence that the number is low, approximately 12–14. Some irregularities in the behavior of the chromosomes in this form were found which have never been seen in any of the other investigated forms. These irregularities were observed in the late anaphases and early telophases of the somatic mitoses. The anaphasic chromosomes pass to the poles rather separated from each other (figure 17). After the poles have been reached they become more closely associated (figure 18). During this time it is possible to observe on the spindle two distinct groups of chromatin structures which form two very regularly arranged rows of small rods. These groups extend toward the periphery of the cell, become transformed into irregular masses, and by the telophase (figure 19) gradually disappear. This peculiarity was observed in somatic mitoses of the different parts of the plant, and roughly resemble the bridges of the lagging chromosomes, which have been described in the meiotic phases in the *Papaver* hybrids by YASUI (1921) and LJUNGDAHL (1922, 1924).

The "Asepalis form" was characterized by the absence of the sepals as the name indicates. The early buds were covered directly by the bright yellow petals. In other respects these mutations were quite normal. The chromosome number of this mutation was the same as in the normal form with the difference (a) that the outlines of the individual chromosomes were not so distinct, and (b) the peculiar shape and size were not so pronounced.

The seeds collected from the two mutating forms were sown and some of the offspring plants have already flowered. The above described characters were transmitted to part of them. The cytological investigation of these forms is not yet completed.

(b) *Forms with different degrees of reduction of the anthers.* As already stated the majority of the progeny of one gynodimorphic × normal plant showed a somewhat intermediate condition of the degree of the reduction of the anthers and of some other characters of the floral structure. For convenience all the forms investigated have been divided into six classes, according to the degree of the reduction of the anthers. From every class are described one or several representatives in which the behavior of the chromosomes was found to be different from that of the other classes, or from the forms of the same class.

The first class (represented in figures 20–25) includes forms with complete reduction of the pollen sacs, namely with purely staminodial flowers in which the staminodia are very small (from 3 to 4 mm). In the second class forms are included in which pollen sacs are absolutely reduced but the staminodia are somewhat enlarged at the tips (figures 26–30). The forms of the third class show some rudiment of the pollen sacs (figures 31–34). Increase in the size of the pollen sacs and of the amount of pollen in these sacs is the outstanding characteristics of the fourth class (figures 35–47). The fifth class includes the forms in which all four pollen sacs are developed, but they still differ in shape and size from the ordinary pollen sacs (figures 48–52). The sixth class includes the forms which have normal pollen sacs.

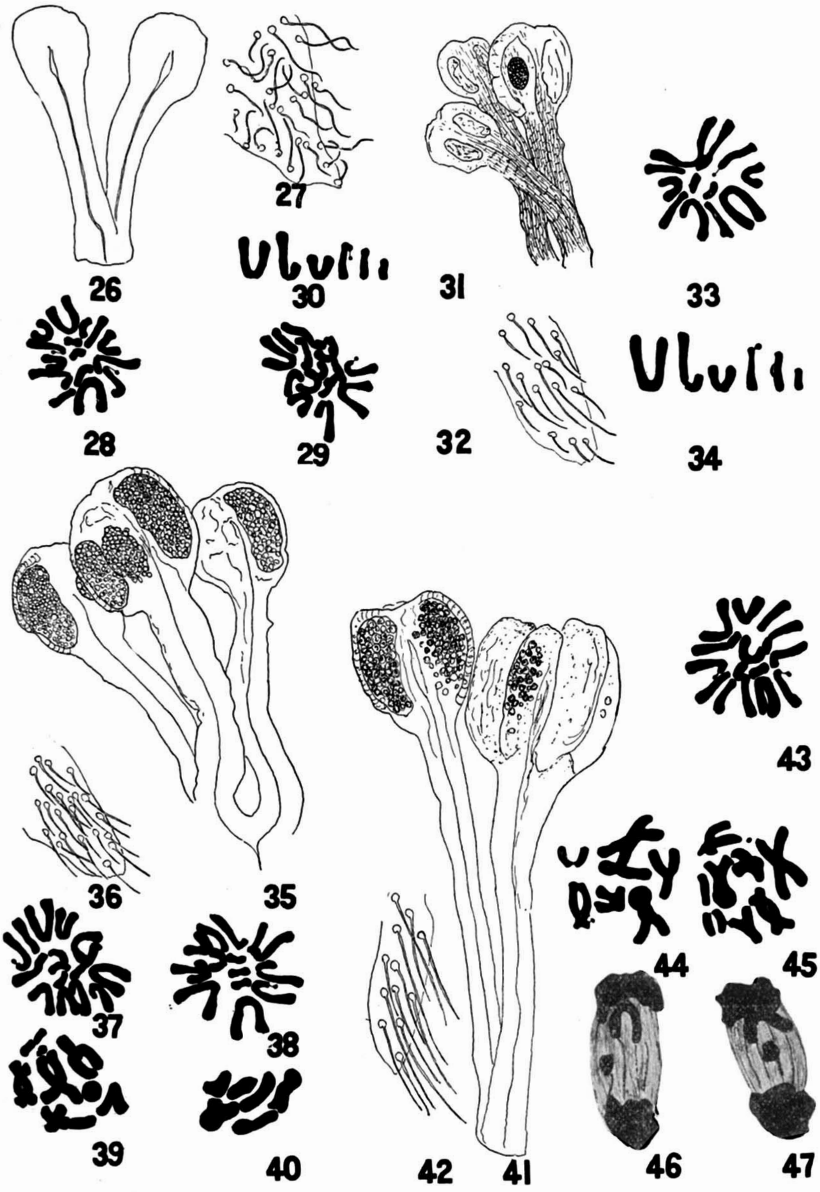
In addition to the other characteristics of the floral structure which were variable in the forms investigated should be mentioned the degree of development of the hairs on the sepals, a feature which is important from the taxonomic point of view.

Class I

Form 1. (Figures 20–23). Petals small of unequal size, varying from 5 to 5.5 mm in length; staminodia very small, without any enlargement of the tips, and without any rudiments of the pollen sacs, beset at the base with a few rather short, straight hairs which are found also on the calyx (figure 21).

Chromosomes 13. It was possible to distinguish the individuality of the chromosomes in the metaphases (figure 22) and to establish the somatic formula (figure 23) $2A+2B+2c+2d''+3e+2f=13$. The two d-chromosomes were provided with satellites of slightly different size. The additional chromosomes were of the rod-type and were of the shape and size approaching the e-chromosomes. In other respects the somatic mitoses of this form did not differ from that of the normal.

Form 2. Plants morphologically absolutely identical with the original gynodimorphic parent were found in this progeny to have 18 somatic chromosomes. As in the mother plant, the chromosomes in this form did not form a triploid complement. The 12 chromosomes could be distinguished by their size and shape, and were identical with the 12 chromosomes of the diploid forms. The 6 chromosomes were of medium size and approached in the shape the chromosomes of the e-type. The exact somatic formula, however, could not be established. It was nevertheless clearly evident that the large chromosomes of the A and B types were present in diploid numbers.



FIGURES—26 to 47.

Form 3. (Figure 24). Petals extremely small (from 3 to 3.5 mm in length; staminodia very small.

This form had the same number of chromosomes and the same somatic formula as form 1.

Form 4. (Figure 25). A form with rather pronounced tendency towards hairiness of the staminodia. Chromosomes 15, somatic formula: $2A+2B+3c+2d''+3e+3f$.

Class II

Form 5. (Figures 26–30). Flowers small; petals from 4 to 4.5 mm; staminodia were somewhat larger than in preceding forms, a little enlarged at the tips, hairs wanting on staminodia; calyx beset with crinkled hairs (figure 27). Chromosomes in the somatic metaphases (figures 28, 29), 15. Formula: $2A+2B+3c+2d''+3e+3f$.

Form 6. In contrast to the preceding, this form had rather large petals ranging from 8 to 8.5 mm in length; the staminodia were identical with those of the preceding form. The chromosomes were not determined.

Class III

Form 7. (Figures 31–34). Flowers extremely small; petals from 3.5 to 4 mm in length. Calyx beset with short hairs (figure 32). Staminodia without hairs; pollen sacs somewhat developed, but the pollen lacking in some, mostly imperfect in others.

Somatic chromosomes, 12, formula as in the normal form, $2A+2B+2c+2d'+2e+2f$ (figures 33, 34). One satellite of the d-chromosomes was established but there was no certain evidence of the other.

Class IV

The forms included in this class all developed abundant but somewhat irregular pollen grains.

Form 8. (Figures 35–40). Flowers of medium size; sepals covered with very abundant straight hairs (figure 36), petals from 5 to 5.5 mm in length. In certain reduced anthers all four pollen sacs developed, in others only three or even two present, and of rather irregular shape (figure 35).

The diploid chromosome number, as determined in the root tips and in the cells of the developing ovary, 15. The arrangement of the chromosomes in two metaphasic plates is shown in text (figures 37, 38). The somatic mitoses did not show any irregularities, and the division of the nucleolus during the early somatic prophases, as has been described as typical for other forms of *R. acris*, was also present.

The reduction division was studied in detail in this form. Only the principal phases, which are directly connected with the distribution of the chromosomes in the gametes will be discussed here. The arrangement of the chromosomes in bivalents and trivalents during the diakinesis occurs from the very beginning of this stage, as contrasted with the facts which were described in the similar stages in triploid *Rubus* and *Crataegi* by LONGLEY (1924a, 1924b).

The diakinesis stage represented in figure 39 shows very distinctly three bivalent and three trivalent sets. The two large chromosomes form the usual large cross, the two B-chromosomes are still distinguished by double crossed shape while the small cross is formed here by the union end-to-end of a third chromosome together with the two bivalents. The d-chromosomes form a bivalent, while the e-chromosomes and the f-chromosomes are quite distinctly associated into trivalents. The e-chromosomes form a ring with a rod connected somewhere to the ring, and the f-chromosomes never cross, and are seldom connected, but simply lie side by side. During late metaphase six sets are present, but it is impossible to distinguish which of them are the bivalents and which are the trivalents, (figure 40). During the anaphase the lagging chromosomes show that the distribution of the bivalents and trivalents is random. The resulting pollen grains receive different chromosome numbers and are of different size and shape. In the somatic mitoses the same peculiar individuality of the chromosomes was established. In most of the preparations studied it was possible to distinguish the two homologous chromosomes of the types A, B and d. The remaining chromosomes of a typical diploid complement were always found to be in the number of three's. Somatic formula: $2A+2B+3c+2d''+3e+3f$.

Form 9. (Figures 41–47). Flowers rather large, petals from 7 to 7.5 mm in length; each anther containing four pollen sacs; filaments very long, without hairs; pollen very irregular in size and shape; sepals with very long straight hairs (figure 42). The stamen to the right in figure 4 has shed the pollen, while in the one to the left the pollen grains are still present. Figure 43 represents a metaphase of the somatic mitoses. The number of the bivalents in the diakinesis of heterotype division was determined to be 6: the remaining chromosomes formed a trivalent. Figures 44, 45 represent diakinesis of the reduction division in the pollen mother cells. The individuality of the chromosomes is clearly distinguished. The A chromosomes always form a very distinct large cross during this stage. The B-chromosomes are distinguished by the typical double cross shape. The c-chromosomes form a small cross, the d-chromosomes are connected at one end and crossed at the other, or they may be open at the other end. The f-chromosomes never cross, and are very rarely connected with each other, forming more or less closely approximated small rods. The e-chromosomes are as usual connected with the nucleolus for a very long time, and associated with them it is possible to observe a third chromosome of the same size (figure 45). In some of the figures, however, the third chromosome is connected with one extra pair (figure 44) which is approximately of the size of the e-chromosomes also, but which is separated from the nucleolus. This third chromosome can lie very close to the extra pair, or it can be connected with it either in the form of a ring with an attached rod, or in the form of a Y-shaped arrangement. Both forms of connection of the trivalents are described for triploid Daturas by BELLING and BLAKESLEE (1923).

During the metaphases the chromosomes shorten but still retain their peculiar characteristics. The anaphases shown in figures 46 and 47 represent the irregularities which have been found in this stage. Lagging chromosomes are present on the spindle.

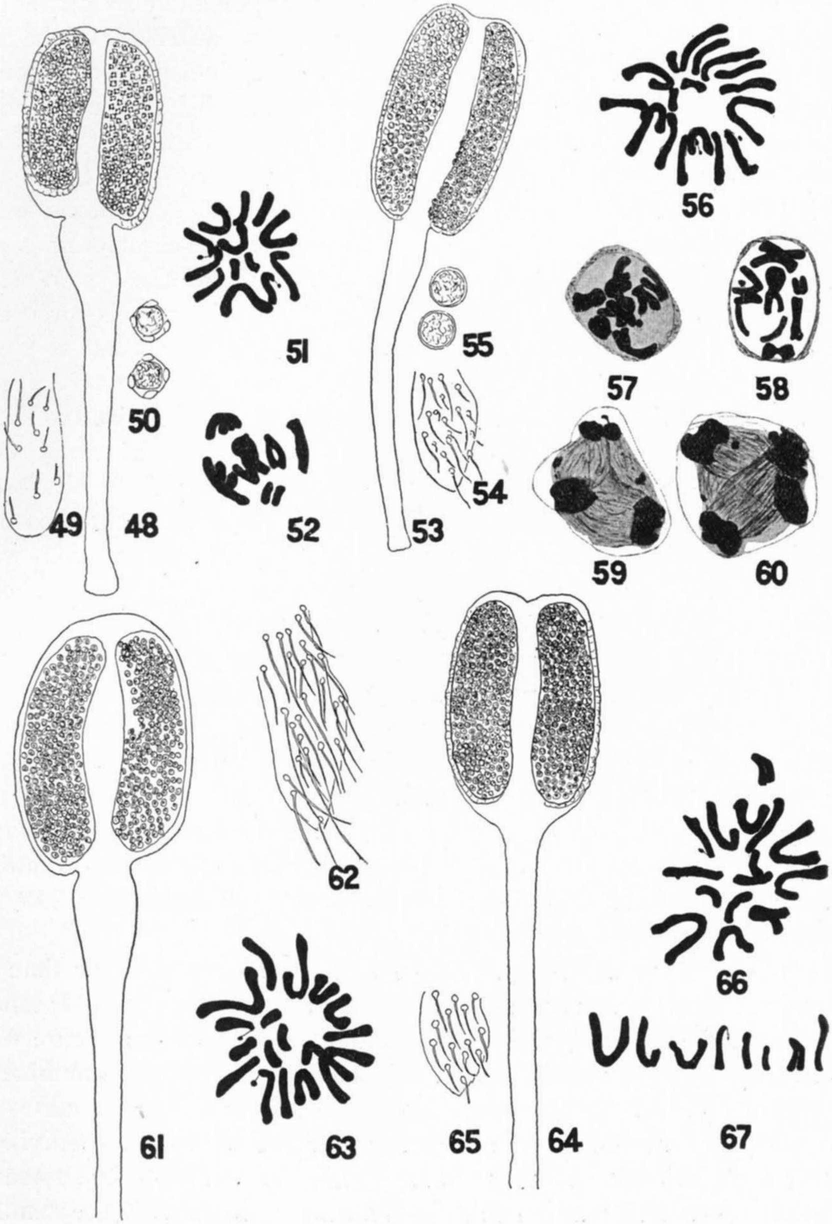
The degeneration of the spindle shows some irregularities. After the chromosomes have reached the poles, the fibers of the spindle thicken and become very pronounced. It is easy to observe that it is made up of small granules, which gradually disappear. The homotype division proceeds comparatively regularly and the resulting pollen grains receive different chromosome numbers as a result of the irregularities during the heterotype division. The large amount of shriveled and irregular shaped pollen grains is an additional illustration of the random distribution of the chromosomes in the gametes.

Taking into consideration the behavior of the chromosomes during the heterotype division, the somatic formula for this form will be: $2A+2B+2c+2d''+3e+2e_1+2f$.

Class V

Form 10. (Figures 48–52). Flowers of normal size, sepals covered with a few short hairs (figure 49); anthers developed almost as in the normal form; pollen sacs only a little shorter, slightly irregular; pollen abundant and apparently normal in function, shape of the grains though very constant, remarkably distinct from that of the normal form. The characteristic quadrangular pollen grains are shown in figure 50. The somatic number of the chromosomes is 13, formula: $2A+2B+2c+2d''+3e+2f$. Two satellites of unequal size are present on the two d-chromosomes of the somatic nuclei (figure 51). Studies of the reduction division showed that the chromosomes in the diakinesis and metaphase of the first mutation division formed regular 5 bivalents and 1 trivalent. Figure 52 gives illustration of this stage.

Form 11. (Figure 53–60). Flowers of normal size; sepals covered with very short but rather abundant hairs, (figure 54) anthers developed almost as in the normal form. The somatic number of chromosomes, 16; the



FIGURES—48 to 67.

outlines of the individual chromosomes sometimes not so regular as in all the preceding forms.

Two satellites were present on the two d-chromosomes. In figures 57–60 are represented the most significant stages in the development of the pollen grains. The figures 57 and 58 represent diakinesis of the first maturation division. There are present 8 very distinct pairs of chromosomes. The A-chromosomes form the large cross, which in the side view can be seen as if one chromosome is overlapping the other. The two second chromosomes of the large size are the B-chromosomes. They are clearly distinguished by their size and peculiar constrictions. The c-chromosomes form the regular small cross in both figures, the d-chromosomes are accompanied by a small body, which is apparently of the type of temporary constrictions. In figure 57 it seems as if this body is connected with the d-chromosomes, whereas in the figure 58 this is not so clear. The e-chromosomes remain connected with the nucleolus for a very long time. Two additional pairs were approximately of the same size and shape as the e-chromosomes. In the figure 58 the chromosomes of one of these additional pairs to the regular complement seems to fail to be closely associated at any rate during the diakinesis. The small f-chromosomes constitute the eighth pair of the complement. No good preparations were obtained from the other stages of the meiotic phase except the telophases of the homotype division. As far as it was possible to judge concerning the distribution of the chromosomes in the gametes from these stages it seems that it was comparatively regular. Lagging chromosomes were not present, the black bodies found in the figures were always the extruded bodies which have been described by several authors in the telephases of the meiotic divisions. The four pollen grains which resulted from the divisions of the pollen mother cells were all of the same size and shape. As a result of the comparison of the behavior of the chromosomes during mitoses and meioses the somatic formula was established as: $2A+2B+2c+2d''+2e+2e_1+2e_2+2f$.

Form 12. (Figures 61–68). Flowers very large, even larger than in the normal form; petals and sepals increased in number from 6–12; sepals covered with very long and very abundant straight hairs (figure 62); petals from 11 to 12 mm in length. The somatic chromosome number is 17 (figure 63) two of the chromosomes are provided with satellites of unequal size. Material for definite conclusions as to the reduction division of this form has not been obtained. From the studies of the somatic divisions, however, it was possible to determine approximately the number of chromosomes and the formula as: $2A+2B+3c+2d''+4e+4f$.

Form 13. (Figures 64–67). Flowers of normal size; anthers apparently normally developed. In the somatic complement some irregularities were found. The form had 15 chromosomes (figure 66); all were very well distinguished by their peculiar shape, size and some individual characteristics. The A-chromosomes were arranged opposite each other, the same position was found for the c-chromosomes, and the e- and the f-chromosomes formed very regular pairs in the metaphasic plate. In the d-chromosomes satellites of different size were present. The three additional chromosomes could not be arranged under any of the categories; two of them apparently formed a good pair and were of the c-chromosome type, the third was very long and rod-shaped.

The following table will summarize the different number of chromosomes which were found in the representatives of the different classes, and sometimes in the representatives of the same class.

TABLE 1

Somatic formulae of the progeny of the gynodimorphic × normal R. acris.

CLASSES AND NUMBER OF THE INDIVIDUALS	FORMULAE	CHROMOSOME NUMBERS
Class I.		
Form 1.	$2A+2B+2c+2d''+3e+2f$	13
2.		18
3.	$2A+2B+2c+2d''+3e+2f$	13
4.	$2A+2B+3c+2d''+3e+3f$	15
Class II.		
Form 5.	$2A+2B+3c+2d''+3e+3f$	15
6.		
Class III.		
Form 7.	$2A+2B+2c+2d''+2e+2f$	12
Class IV.		
Form 8.	$2A+2B+3c+2d''+3e+3f$	15
9.	$2A+2B+2c+2d''+3e+2e_1+2f$	15
Class V.		
Form 10.	$2A+2B+2c+2d''+3e+2f$	13
11.	$2A+2B+2c+2d''+2e+2e_1+2e_2+2f$	16
12.	$2A+2B+3c+2d''+4e+4f$	17
Class VI.		
Form 13.	$2A+2B+2c+2d''+2e+2f+3$ (extra)	15

The above table affords the basis for the following conclusions:

(a) Different chromosome numbers are found in forms belonging to the same class. The gynodimorphism was found in *Ranunculus acris* in the forms with different chromosome numbers. In form 13, where the morphological characters were absolutely identical with the normal form, the constitution of the nuclei was different.

(b) One and the same number of chromosomes, and even one and the same somatic fomula, were found in forms with every pronounced morphological differentiations. For instance, 15 chromosomes have been found in the forms of the first, second, fourth, and sixth classes. Further, the somatic formulae of form No. 4 of the first class, and that of form No. 8 of the fourth class were absolutely identical, although the morphological characters were different.

(c) There is no correlation between the number of chromosomes and the degree of reduction of the anthers. Both high and low chromosome numbers were found among the forms of the first class. The morphological differences between the forms of the first class having 18 chromosomes and that with 13 somatic chromosomes were very insignificant.

The somatic formulae which were established on the basis of the behavior of the individual chromosomes show that in most of the forms discussed the additional chromosomes formed trivalents during the heterotype division, and were distinguished as being triple mates of some chromosomes of the somatic complement. In one case, however, the diakinetic chromosomes formed instead of several bivalents and trivalents, eight distinct pairs, which later behaved as absolutely normal bivalents. In two cases on a row with some trivalents, there were present quite distinct and new additional bivalents. The presence of the trivalents in the meiotic nuclei is a very strong evidence for the theory of the origin of those forms from the crosses of the polyploid forms of *R. acris*. The strong affinity of the homologous chromosomes seems, however, in some forms to lose its efficiency and we find there instead of the trivalent new bivalents as in form 11 with 16 chromosomes.

As to the individual chromosomes which are involved in the variations, it is interesting to emphasize that the A and B chromosomes have never been found in triple numbers. Apparently the chromosomes of the d-type behave in a similar way. The remaining chromosomes are all found in triple numbers in different forms, but most commonly the e-chromosomes figure as the varying members. In almost all forms satellites have been found. In the majority of cases two satellites were present in every nucleus. In some of the forms not very definite evidence was found concerning the second satellite. Possibly it was absent and we have an example of the asymmetric races of S. NAWASCHIN (1912).

Different results were obtained by BELLING and BLAKESLEE (1922) in investigations of the progeny of the triploid Daturas pollinated by diploid. The plants with one extra chromosome showed that this extra chromosome could be of the extra large, large, medium, small or extra small types.

DISCUSSION

It is evident from the above observations that gynodimorphism in *R. acris* is associated with somewhat unstable conditions of the nuclei, a condition to which BLAKESLEE (1921) gives the name of "unbalanced changes." A similar condition is typical for the hybrids between different species and for the progeny of the crosses between the polyploid races of the same species.

JEFFREY (1918) found a considerable proportion of imperfect pollen in *R. acris* and explained this condition as a consequence of previous hybridization. Theoretically both these conditions are quite likely to have taken place in the cases under discussion. But for *R. acris* from Russia direct evidence points rather strongly to the probability that the crosses between different polyploid races have played a more important role in the production of gynodimorphism than general polymorphism.

The first evidence against the interspecies hybrid nature of the forms under consideration is that so far as known hybridization between the species is not recorded to result in the degeneration of the stamens to the degree of staminodia. It is true that generally there is a tendency towards degeneration within the anthers in some of the hybrids as TISCHLER (1906) has described for Bryonia. LONGLEY (1924b) considers similar degeneration within the anthers of *Crataegus Smithii* as evidence for the hybrid nature of this species.

The second evidence against hybridization (having taken place in the modern times) as the cause of gynodimorphism in *R. acris* from Russia is that the only other species of Ranunculus found in flower at the same time with *R. acris* on the meadows and lawns at Czarskoe Selo, was *R. repens* L., a species with very pronounced morphological distinctions. Doctor DOMIN of Prague, a specialist of the *R. acris*×*repens* hybrids, examined my material and concluded that it does not resemble at all the known hybrids of the above mentioned species.

There is still another possible theoretical explanation of the origin of the gynodimorphic condition in *R. acris*, which could be the result of hybridization and subsequent segregation which may have taken place in the far past. The typical arrangement of the chromosomes during meiosis in the hybrids between different species is that of the formation of the bi- and uni-valents, as described by ROSENBERG (1909). It is true that in a recent investigation LJUNGDAHL (1924) showed for Papaver hybrids the possibility of the associations of the chromosomes in pairs independent of their origin from different parents, or from the same parent. He calls this phenomenon autosyndese. Similar evidence was brought forward by HAASE-BESSEL (1921).

The study of the individuality of the chromosomes offers some possibilities for distinguishing the chromosomes and shows the occurrence of the peculiar triploid sets of certain chromosomes in the investigated progeny. All this furnishes good evidence for the theory (ERNST 1922; OVEREEM 1921, 1922; MOL 1921) which would explain the polymorphism in *R. acris* as a result of crosses between polyploid races and subsequent segregations. The gametes obtained as a result of similar crosses and fertilized by normal, or by modified ones are in their turn the cause for the further variations in subsequent generations. On the other hand they also can give rise to some forms, like the above described from 11, with 16 somatic chromosomes, in which the chromosomes will pair during the diakineses and metaphases, and the resulting gametes will have a constant chromosome number, different from that of the normal. Here we see an example in which the unbalanced conditions of the nuclei become balanced and results in the origin of a form with a new chromosome complement. It will be remembered, that the morphological characters of this form were slightly different from the normal form.

While carrying on the above investigations it seemed of interest to compare the plants of these cultures with the races of *R. acris* grown wild in America. With this purpose in view material of *R. acris* was collected from the meadows of the BOTANICAL GARDEN, Bronx Park, N. Y. Studies of the reduction division of this form is described separately (SOROKIN 1926). Here it is only necessary to note that this form has 14 chromosomes, which form 7 very distinct pairs during diakinesis, and that later, after the homotypic division is completed, the resulting pollen grains receive 7 chromosomes each. The divisions so far as studied appeared to be perfectly regular.

HOCQUETTE (1922) studying the number of chromosomes of *Ranunculus acris* L. subsp. *boreauanus* (Jord) Rouy et Fouc. from France found it to have 16 somatic chromosomes. For *Ranunculus repens* L. var. *typicus* Beck. which formerly was reported as having 24 chromosomes, HOCQUETTE (l.c.) gives 32 as the diploid number, and explains that the difference between the results of the investigators is due to the fact that the different varieties and races of the same species may have different chromosome numbers. The same condition was found by the same author in another representative of the Ranunculaceae, namely in *Thalictrum minus*, in which there were previously reported 24 diploid chromosomes, whereas HOCQUETTE (l.c.) gives the number as 48.

LITARDIÈRE (1921) pointed out that in very closely related species, and even in the varieties of the same species, the number of chromosomes can be quite different. Further, either the varieties of a certain species with different chromosome numbers can differ morphologically, or on the contrary they can be absolutely identical as to their morphological characters. As examples of these statements LITARDIÈRE (1921, 1922) mentioned the following plants which have different cytological strains: *Salvinia natans*, *Pteris tremula*, *Podophyllum peltatum*, *Senecio vulgaris*.

It is quite probable that the races of *R. acris* the one from the BOTANICAL GARDEN, Bronx Park, N. Y., with 14 chromosomes in the somatic nuclei, and the other from Czarskoe Selo, Petrograd, with 12 diploid chromosomes, which are absolutely similar morphologically, represent an example of cytological races or strains in *R. acris*.

SUMMARY

1. Gynodimorphism in *Ranunculus acris* is associated with unstable condition of the plant characters and is an indication of the unbalanced types of changes which have taken place in the chromosome complement of the nuclei.

2. The progeny of a gynodimorphic plant by normal shows great variations in the morphological characters associated with the changes in the chromosome numbers. In the meioses in these forms irregularities are shown in the distribution of the chromosomes, and the resulting gametes receive different chromosome numbers. The union of the gametes with the modified chromosome numbers with the normal gametes or with the modified ones, is found to be responsible for the further changes of the chromosome constitution in the later generations and for the changes in the morphological characters.

3. The individuality of the chromosomes is found to be very distinct. The chromosomes in all investigated forms are distinguished according to their shape, size, presence of satellites and constrictions.

4. The presence of the trivalents in the progeny of the gynodimorphic plant by normal suggests that the unbalanced condition of the nuclei in these forms is a result of the irregular distribution of the chromosomes

during the meiotic phases, similar to that of the progenies of the polyploid forms crossed by normal or by other polyploids.

5. The occurrence of the different chromosome numbers in morphologically identical plants is an indication of the occurrence of cytological races in *R. acris* L.

6. Generally polymorphism in *Ranunculus acris* might be explained as a result of crosses between different polyploid forms and segregations taking place in the subsequent generations.

7. The unbalanced type of changes in the nuclei of the progeny of the polyploid forms by normal can be the beginning or initiation of the balanced types with different chromosome complements.

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