

CROSS BREEDING LUPINES

(TECHNIQUE)

MINOR THESIS IN PLANT BREEDING.

Submitted by

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## Cross Breeding Lupines.

### Introduction.

The work of which this paper purports to give an account, was done as the practical part of a course in Plant Breeding offered by the writer as a part of the work for the degree of Master of Science.

The subject of plant breeding has claimed a great deal of attention from both scientists and practical plant breeders in the last few years, and while much progress has been made, no field of investigation is more inviting today. This paper deals with the description of the plants used and the technique of the work; the results to be written later when the F<sub>2</sub> and F<sub>3</sub> generations have been grown.

Work was begun in the fall of 1908, but the only result of the session of 1908-09 was the experience gained. Though several mistakes were made which caused the work of this session to be a failure so far as results were concerned, two were primarily responsible. The first was an attempt to transplant the lupines while small. Not a single transplanted lupine lived, although all the precautions usual in such cases were observed. Subsequently no attempts have been made to reset the plants. An old, well established plant is seriously injured if any considerable number of its roots are disturbed. The second cause of the negative results of the first year was the failure to sterilize the soil in which the plants were grown. As a consequence of this, all the plants were killed by a wilt, most probably caused by a *Fusarium*. The small red spider, *Tetranychus bimaculatus* Haw, which is sometimes a serious pest in greenhouses, was also very detrimental to the healthy growth of the

plants. It was learned, however, that it could be controlled by thoroughly syringing the plants with cold water, taking care not to wet the bed. The precautions taken to prevent or overcome these difficulties in subsequent efforts are noticed later.

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## The Genus *Lupinus*.

The genus *Lupinus* belongs to the family Leguminosae, or pea family. The name lupine is derived from the Latin word *lupus*, meaning wolf. This name is said to have been given them because they were supposed to impoverish the land- a strange assumption since they are nitrogen gathers.

Wyman, in the Cyclopedia of Horticulture, states that there are about 80 species, while Britton and Brown, in their Illustrated Flora of the Northern States and Canada, place the number of species at more than 100. The larger number are found in Western North America, a few grow in the Mediterranean region, and Gray's New Manual of Botany gives one species, *perennis*, as indigenous to Eastern North America. One species, *arboreus*, is shrubby; some are herbaceous perennials, but most are annuals.

They are used for ornamental purposes, for fodder, and as soil improvers. As ornamental plants, they are useful for borders, bedding and cut flowers. As feed, some of the less coarse growing species may be fed either green or as hay. The mature seed contain an alkaloid which is dangerous to stock. The greatest economic importance of lupines is as soil improvers, especially on light soils. While they cannot be grown successfully on soils containing much lime, when inoculated, they make a very heavy growth on other types of soil. They are used for plowing under to improve the land in the Mediterranean region and the Pacific coast states, but it is doubtful if they can be used to any advantage in any other section of our country because soy beans, cow peas, clovers and other leguminous plants answer the same purpose.

Description of species and Varieties Grown.

In the fall of 1909 seed of the following lupines were secured from J. M. Thorburn & Company of New York:

*Lupinus Cruckshanksii* A. Gray: *Lupinus luteus* L.; *Lupinus hirsutus* L. (blue); *Lupinus hirsutus* L., Var. *ruber*, Hort., and *Lupinus hirsutus* L., Var. *albus*, Hort. As grown in the greenhouse, the various species and varieties may be described as follows:

*Lupinus Cruckshanksii* is a native of South America. It is strikingly different from either of the other species grown. Compared with *hirsutus*, it is a more upright grower, and taller-attaining a height of 6 or 7 feet. While a luxurious grower, it is not as hardy as either *hirsutus* or *luteus*. The stem is covered with a white bloom when young and finally becomes glabrous. The compound leaves have a petiole 3 to 4 inches long, and bear from 8 to 10 narrow- oblong, blunt pointed leaflets palmately arranged. The leaflets are from 2 to 3 inches long. The stipules are small, linear acuminate, and deciduous. The inflorescence is determinate. The flowers are borne in long interrupted racemes, the verticillate arrangement of *luteus* not being present. The flowers are large, about an inch in breadth, and very showy. There are two types of flowers as to color. The second type did not occur in 1909-1910, hence the crosses made were without reference to it. The predominant color of the first type is dark blue. When the flower first opens the standard is blue with a yellow center, as the flower grows older, the yellow changes to a reddish brown becoming quite dark before falling away. The wings are light blue at first becoming somewhat darker with age. The keel is white except for

the tip which is slightly tinged with blue. The predominant color of the second type is white. When the flowers first open they are cream white except the center of the standard which is light yellow becoming brownish yellow before falling away. The sides of the standard and the wings turn pale lilac (the wings paler than the standard) with numerous white blotches, some of which have a dark brown eye. The keel also becomes pale lilac. A raceme bears from 4 to 12 pods containing from 4 to 7 seed each. The oldest flowers <sup>only</sup> set pods. The pods are flattened, slightly constricted between the seed, and are covered with short, soft, white hairs. The seed are rather small- hardly as large as a garden pea- flattened, smooth and white.

*Lupinus luteus* is a low growing, branching plant from 1 1/2 to 2 feet high. The stem, leaves, and pods are hairy. As seen with *Cruckshanksii* and *hirsutus*, it seems to be a very leafy and compact plant. The digitately compound leaves have petioles from 3 to 5 inches long and bear from 7 to 10 spatulate leaflets. The inflorescence is determinate. The flowers of the raceme are arranged in from 5 to 9 whorls, each whorl with 5 flowers. The flowers are rather small; the standard being from half to three fourths of an inch high, and from three-eighths to half an inch broad; the wings are half an inch long and three-eighths of an inch broad, aduate near the tip: the keel is sythe shaped, and white except the tip which is dark blue. As the name would indicate, the flowers (standard and wings) are lemon-yellow becoming slightly darker with age. The flowers have a very rich and agreeable odor. The pods, from five to ten of which set on each raceme, are from one and one-half

*to two and one half*

inches long, flattened and slightly constricted between the seed which vary in ~~number~~ from three to seven. The seed are small, hardly as large as those of *Cruckshanksii*, flat, and have a distinct soapy feeling. The color is a dull, mottled brownish-gray.

*Lupinus hirsutus* is one of the annual species like the two just described. It is commonly known as "blue lupine," the flowers of the plants found growing wild being of this color. The stem, leaves, bracts and calyx are covered with soft silvery hair. As grown in the greenhouse, they have reached a height of three feet or even more, and are much branched. The digitately compound leaves have petioles from four to six inches long. The leaflets are from seven to eleven in number, oblong-spatulate in shape, and from one and one-half to two and one-half inches long. The stipules are linear-lanceolate, and deciduous. The inflorescence is determinate. The flowers of the raceme are usually verticillate, though sometimes scattered. Five is the normal number per whorl. The flowers are dark blue in color except for the center of the standard which is white and occasionally a white spot, twice the size of a pin head. The standard, like that of both the species described above, is strongly reflexed, an inch and a quarter long and an inch broad. The wings are three-fourths of an inch long and three-eighths of an inch broad, cohering below. The keel which is white, except for the dark blue tip, is sythe-shaped and about an inch long. The flowers

have a rather unpleasant odor. From three to six of the oldest flowers of each receme set seed. The pods are broad, flat, hairy and constricted between the seed of which there are from two to four. The seed are about three-eighths of an inch broad, flattened, and rough with a light flesh-colored ground color, spotted with brownish-red and a darker spot covering about one-third of each side one end terminating near the hilum. The seed coat is very tough and it is often necessary to remove it from the large, plump cotyledons when they emerge from the soil

Variety albus is like the blue in every way except that the flowers are solid white. While the plants were larger than those of either the blue or the red variety, it is believed that local environment and not any inherent difference accounts for this.

Variety ruber is not different from the blue and white varieties except as the name would indicate. The wings and standard are rose red in color except for the center of the standard which is white.

#### The Bed.

The south end of the bed in the middle greenhouse was used in both 1909-10 and 1910-11. The plants were grown in ordinary six inch pots which were plunged in the bed. The bed is about four feet wide and eight feet long. In 1909-10 thirty pots placed in squares were used: in 1910-11 thirty-two pots were used, placed in equilateral triangles. This would be too close if every pot grew a plant to maturity.



### Treatment of the Soil.

On account of the wilt of the first year, the soil was sterilized before planting the seed. The bed was first thoroughly spaded up so as to aerate the soil. After filling the pots, which were to be sterilized separately, the bed was thoroughly saturated with boiling water, it having been allowed to become quite dry. The pots were carefully filled and watered before being placed in the autoclave which held only three. The first year, 1909-10, the pots were subjected to a temperature of 120 degrees Centigrade (30 lbs. pressure) for fifteen minutes; the second year they were kept under twenty pounds of pressure for thirty minutes. The soil is no doubt changed by this process. The physical, chemical and biological conditions are altered. In some of the pots the capilarity of the soil seemed to be broken up and this, perhaps, accounts for the slow germination of the seed in some cases, though it is believed that the seed germinate unevenly. Russell and Hutchinson explain the "Contribution Laboratory Rothamsted Experiment Station, 1909, pp. 111-114."

chemical and biological changes which take place in soil treated in this way. The plants seem to start off slowly but later they grow more luxuriantly than those grown in untreated soil.

### Treatment of Seed.

The seed were treated with ten per cent formalin for fifteen minutes in the first case, and in a solution of bichloride of mercury (one to five hundred) for two minutes in the second case. On account of the thinness of the seed coat of Cruckshanksii its seed should not be kept in this strength of formalin so long.

In order to hasten the germination of the seed, they were soaked for twenty-four hours, then treated and immediately planted.

### Planting the Seed.

The seed bed and seed having been prepared, as described above, from two to four seed were planted in each pot. It would be better to plant only one seed in each pot, because the plant soon sends its tap root through the hole in the bottom of the pot and if the others are confined therein they are soon choked out by their more fortunate neighbor. The seed were not covered very deeply since they, like the bean and some other plants, lift their cotyledons from the ground.

### Growing the Plants.

Only two plants were left in each pot in most cases, and perhaps it would have been better to have left only one. The watering of the plants was done carefully. Ordinarily one good watering every ten days or two weeks was sufficient. The plants were never given any shade, though no doubt, this could be done to advantage at times. The usual precautions in regard to cultivating were observed. A complete commercial fertilizer was applied: first when the plants were two or three inches high and again as they were coming into bloom.

The flowers begin to come about three months after planting the seed and continue for six months. The seed of *Cruckshanksii* and *hirsutus* mature in ninety to one hundred days, those of *luteus* in about sixty days from polination.

### Gathering and Storing Seed.

When mature, the seed were gathered and those of each pod were placed in an envelope which showed their parentage and time of maturity together with any remarks. For convenience in keeping a record, each raceme was given a letter and each pod a numerical designation. It may be said here that only one kind of pollen was applied to the flowers of a raceme, hence A - 1, A - 2, A - 3, were of the same parentage.

### Tools used in Emasculation, Depollination and Pollination.

A small pair of scissors is necessary to cut the keel at the base when removing the stamens and pistil from it in securing the pollen. They are again used to cut off the tip of the keel as described elsewhere. While little used, the scissors are, nevertheless, indispensable. The blades need not be more than half an inch in length.

Light, sharp pointed forceps are used in removing the anthers. Straight pointed ones may be used almost, if not quite, as well as those with curved points- the important point being their fineness. They are also used in placing the pollen on the stigma.

A fine, sharp pointed needle is used in exposing the stamens and pistil. It may be either round or flattened at the end.

A good hand lens which magnifies from five to ten times is useful, if not essential, and it will sometimes enable the operator to use a flower that he would otherwise throw away for fear it might not be all right.

For depollinizing the flowers an ordinary atomizer was used. The only objection to this type of syringe is its unhandiness. A simple rubber bulb with a fine bore-glass tube would be more convenient, and perhaps would do the work as well.

The camel's hair brush and glass for applying and holding the pollen respectively, which are usually found in a kit for crossing plants are not needed on account of the method and time of the application of the pollen.

Tags of thick, white paper were used on each raceme cross pollinated. These tags were about one inch wide and two and one half inches long. They bore the name of both the maternal and paternal parent together with the date the cross was made. Only one date was given, this being the same for emasculation, depollination and pollination.

#### Technique of Crossing.

In December, 1909, the lupines planted the previous October began to bloom. An attempt was at once made to cross the white and red varieties which were the earliest. Very soon after the white of the standard began to show through the lips of the calyx the floral envelopes were removed as carefully as possible with a small pair of scissors and the anthers cut or pinched off. The pistil was now left with no other protection than the bract- which was almost none- for forty-eight hours or longer, the time necessary for the stigma to reach that stage of development where it was in a receptive condition for pollen. In the meantime, pollen from the paternal parent was collected in a small glass dish and held until needed; it was then placed on the stigma of the emasculated flower with a camel's hair brush. Of twenty-five or thirty

flowers, white and red, thus emasculated and pollinated not a single one set seed. It now became necessary to use some other method for making the crosses required to carry on the work.

The first change it was thought wise to make was to remove the anthers without mutilating the flower as much as had been done. By allowing the flower, or bud, to become twelve to twenty-four hours older, it was found possible to leave the calyx intact and to cut off only about the upper half of the standard, wings and keel instead of cutting off almost the entire floral envelope. The pollen was applied to these stigmas with a camel's hair brush as before. A few pods, manipulated in this way, set, but the percent was too low, and it was thought necessary to still further perfect the method if possible.

It was now found possible to remove the anthers without cutting away any of the flower if it was allowed to reach a little more advanced stage of development. If the anthers had dehisced, the flower was rejected, but it was also found that the anthers were often opened in removing them with the result that more or less pollen was left on the stigma. To throw away all these flowers meant a great waste of time and material, and in a short while if only a very small amount of pollen got on the stigma it was blown off, but if a large amount got on the stigma, and well down among the hairs covering it, blowing was unsuccessful. One day about this time, an atomizer which had been used just before in some other work, was noticed setting close at hand, and the thought at once occurred that it might be used to do the blowing. From this time on it was used to wash off the pollen whenever the anthers were ruptured in removing them. After a while a sufficient degree of skill was attained to make the use of the atomizer seldom necessary, but it is always available when needed and saves flowers that otherwise would have to be rejected.

In the meanwhile, it was found that the method of emasculating and depollinating were much better than the method of applying the pollen. By the method above mentioned, it was not only difficult to apply the pollen but very wasteful of it. Besides the stigma was exposed thus causing it to dry out, the pollen was easily shaken off, and other pollen might get on it. To overcome some of these difficulties, a small piece of paper or leaf was fastened over the flower. This, however, was unsatisfactory because it was troublesome to put on, did not stay on well, and was ineffective. In an attempt to imitate nature as closely as possible, it was found that the difficulties of the pollination had been surmounted. The stigma of the lupine remains within the tip of the keel until after fertilization when with the development of the seed incident thereto, it is forced through the tip. The stamens are shorter than the pistil in the young bud, but as the bud develops, their filaments lengthen, and at the time the anthers open the stigma, in the tip of the keel, is surrounded by them. The stigma is thus enclosed in a mass of pollen, and is protected from drying out and contamination with foreign pollen by both the keel and the surrounding pollen mass.

As described in detail further on, the tip of the keel containing its pollen is placed over the stigma just as it would be if left on its own stigma. This method supplies an abundance of pollen, prevents the drying out of the stigma, and protects it from any foreign pollen. Another point in favor of this method of applying pollen is the small amount required. While it is true that one flower produces enough pollen to fertilize a number of stigmas, it was a fact, nevertheless, that in practice it did not fertilize one, because of the waste in gathering and applying in the usual way with a camel's hair brush.



This method of crossing lupines has been eminently successful, only about five percent of the crosses failing to set pods. The method was perfected before Oliver" published his work of a similar nature. Nothing new or radically different from ordinary methods is claimed for it, but it is believed to be a modification well adapted to the flower upon which it was used, and it may suggest special methods well adapted to other plants.

#### Emasculation and Depollination.

##### Size of Flower to Emasculate.

The object is to catch the flower just before the stamens open. At this time, the flower is about half an inch long, and about one-eighth or three sixteenths of an inch of the unopened standard is showing above the calyx. The keel may not be showing at all or only very slightly.

To emasculate the flower: one in the proper stage of development having been selected; with the thumb and fore finger of the left hand grasp the tip of the standard and wings, being careful not to grasp the tip of the keel and the enclosed pistil and stamens; and run the needle down the side of the flower pressing down the lower lip of the calyx where it will remain out of the way on account of its trough like shape; insert the point of the needle between the wings and the two margins of the unopened standard near the base and to the right side of the keel, and with a careful upward movement pull apart the wings and the yet cohering sides of the standard; now by turning the point of the needle to the left, the tip of the keel is lifted out of and to the left of the wings and standard. The thumb and fore finger of the left hand are now slipped

further down the flower, the keel resting between them. Insert the needle between the cohering members of the keel at or near the base and draw it upward opening the keel, at the sametime being careful not to injury the pistil or disturb the anthers. With a little practice, this can be done successfully in almost every case. The stamens are now exposed to view and the anthers are removed with a pair of fine pointed forceps. As much of the filiments should be left as possible. With a hand lens, carefully examine the stigma, where a single grain of the yellow pollen can easily be seen among the white hairs. The pollen is sticky at this stage and generally coheres in quite large masses. If any pollen, or the suspicion of it is detected, it is washed off with a syringe of some kind. If the anthers have opened the flowers are always rejected, but if they are opened by the operation of emasculation they are **always** used, for it is not possible for the pollen tube to enter the stigma in the short time before it is washed off with the water from the syringe.

#### Pollination.

As soon as the flower to be used as the maternal parent is emasculated and depollinated the pollen is applied. This is done immediately for three reasons. First; it saves time and reduces to a minimum the chance of overlooking any emasculated flowers; second; it prevents the drying out of the stigma; and third; it prevents any foreign pollen getting on the stigma. Sometimes pollen is applied a second time, the following day, though this is most probably unnecessary.

About forty-eight hours after the standard is fully reflexed the flowers are in the right stage of development for furnishing pollen. At this time the pollen has been almost completely shed by the anthers which



are slightly withered and thus easily withdrawn, as described below. The keel is still almost completely closed, the pistil not yet having projected through the tip. The flowers near the top of the raceme are used for this purpose since they will not set seed and consequently cannot be utilized in any other way. The oldest flowers of each raceme are used as maternal parents.

A flower in the state of development described above is pinched or cut off close to the stem. It is then grasped at the base of the calyx between the thumb and fore finger of the right hand, and, without disturbing the keel or the contained organs, the wings and standard are pulled off with the thumb and fore finger of the left hand. Now grasp the keel firmly just below the anthers with the thumb and fore finger of the left hand and clip off each side of the keel with the scissors, being careful not to cut the stamens tube and pistil. Now while holding the keel firmly with the thumb and fore finger of the left hand, grasp the flower at the joint of insertion of the corolla and draw the stamens and pistil from the keel. If a flower in the proper stage of development is chosen almost all the pollen is left in the tip of the keel when the pistil and stamens are withdrawn. Now lay the keel in the palm of the left hand and cut off about three sixteenths of an inch of the tip. This length of tip is best; it holds all the pollen, is easily placed on the stigma, and stays on as well as a longer one would. Taking the forceps, used in pinching off the anthers of the emasculated flower, grasp the tip of the keel about the center of the edges that cohere and carefully place it over the stigma of the emasculated flower. It should be pulled down until the pollen is beginning to be forced out at the tip. This will insure the

stigma being in contact with the pollen, and also leave it protected from drying out and from foreign pollen. This tip soon withers and seals in the stigma, as it were.

### Crosses.

The following table shows the crosses attempted together with their differential characteristics and the results.

<u>No.</u>	<u>Crosses</u>	<u>Characteristics</u>	<u>Maternal Parent</u>	<u>Paternal Parent</u>	<u>Result.</u>
1	L.h.a.X L.h.b.	Flower color	White	Blue	Successful
2	L.h.a.X L.h.z	Flower color	White	Red	"
3	L.h.a.X L.C.	Flower color	White	Blue	Negative
4	L.h.a.X L.C.	Foliage surface	Hairy	Smooth	"
5	L.h.a.X L.l.	Flower color	White	Yellow	Doubtful
6	L.h.a.X L.l.	Growth habit	Tall	Low	"
7	L.h.b.X L.h.a.	Flower color	Blue	White	Successful
8	L.h.b.X L. h.x	Flower color	Blue	Red	"
9	L.h.b.X L.C.	Standard color	White center	Yellow center	Negative
10	L.h.b.X L.l.	Flower color	Blue	Yellow	"
11	L.h.b.X L.l.	Seed coat	Rough	Smooth	"
12	L.h.z.X L.h.a.	Flower color	Red	White	Successful
13	L.h.z.X L.h.b.	Flower color	Red	Blue	"
14	L.h.z.X L.C.	Flower color	Red	Blue	Negative
15	L.h.z.X L.C.	Seed color	Flesh color with: red & v.d. red spots	White	"

No.	Crosses	Characteristics	Maternal Parent	Paternal Parent	Result.
16	L.h.r.X L.C.	Seed coat	Rough	Smooth	Negative
17	L.h.r.X L.l.	Flower color	Red	Yellow	Doubtful
18	L.h.r.X L.l.	Seed color	Dun	Flesh color with: red and very dark tinted spots	"
19	L.C.X L.h.a.	Flower color	Blue	White	Negative
20	L.C.X L.h.a.	Foliage surface	Smooth	Hairy	"
21	L.C.X L.h.b.	Standard color	Yellow center	Blue	"
22	L.C.X L.h.r.	Flower color	Blue	Red	"
23	L.C.X L.h.r.	Seed color	White	Flesh color with: red & very dark red spots	"
24	L.C.X L.l.	Flower color	Blue	Yellow	"
25	L.C.X L.l.	Foliage surface	Smooth	Hairy	"
26	L.C.X L.l.	Seed color	White	Dun	"
27	L.C.X L.l.	Growth habit	Tall	Low	"
28	L.l. X L.h.a.	Flower color	Yellow	White	"
29	L.l.X L.h.a.	Growth habit	Low	Tall	"
30	L.l.X L.h.b.	Flower color	Yellow	Blue	Doubtful
31	L.l.X L.h.b.	Seed coat	Smooth	Rough	"
32	L.l. X L.h.r.	Seed color	dunn	Flesh color with: red & very dark red spots	"
33	L.l.x L.h.r.	Flower color	Yellow	Red	"
34	L.l.X L.C	Flower color	Yellow	Blue	Negative
35	L.l.X L.C.	Foliage surface	Hairy	Smooth	"
36	L.l X L.C.	Seed color	Dun	White	"
37	L.l X L.C.	Growth habit	Low	Tall	"

Result of Crossing.

The only undoubtedly successful attempts at crossing were those between the different varieties of hirsutus. In the other crosses, there were some mechanical difficulties to overcome on account of the difference in size of the flowers and because of the openness of the tip of the keel of Cruckshanksii, but it is not believed that these were of such a nature as to cause complete failure. It is more likely that the different species of the genus Lupinus do not hybridize freely, at least, the three worked with did not. These crosses will be attempted again. Only the results of the F<sub>1</sub> generation are available and the F<sub>2</sub> generation may necessitate a change of opinion but it is believed that Meadel's law of inheritance will apply.

Cross Number One.

L.h.a.♀ X L.h. (b)♂

This cross was made to see which law of inheritance, blended, mosaic, or alternative, applied in regard to flower color. Three F<sub>1</sub> plants were grown and the flowers of all were so near alike that a description of one applies to all. The plants were not very vigorous but set a number of pods. The flowers upon opening, are light blue except for a patch of white along the entire lengths of the center of the standard. This white center is quite well defined and appears to be pure white, except sometimes a few dark views, when the flower opens. A border 1/24 of an inch wide along the edge of the standard is white or very light blue when the flower opens but this very soon becomes darker. The white center of the standard gradually turns red. The wings are light blue, gradually becoming darker and finally assuming a reddish tinge.

The tip of the keel is dark and seems to be just the same as paternal parent.

Cross Number Two.

L.h.a.♀X L.h.1.♂

The object of this cross was to test the inheritance of flower color. Six of these plants were grown and they were among the most vigorous in the entire bed. The flowers of all the plants were almost identical in every way- differing no more than individual flower on the same plant. The description of the flowers of cross number one applies to the flowers of this cross, except that the red tinge began to show a little earlier and was a little darker.

Cross Number Seven.

L.h.(b)♀X L.h.a.♂

It will be noticed that this cross is a reciprocal of cross number one, the object therefore being the same. Only two plants were grown. The color of the flowers seemed in every way identical with those of the reciprocal cross.

Cross Number Eight.

L.h.(b)♀X L. h.1.♂

Five plants of this cross were grown the object of which was to study the inheritance of flower color. These flowers were identical on all the plants, and, while showing the same general marking as those of cross number one, were a shade darker in color than those of cross number one or cross number seven.

Cross Number Twelve.

L.h.z.♀ X L.h.a.♂

The four plants of this cross illustrate the dominance of blue in the F<sub>1</sub> generation. Every plant bore the same characteristic flowers described in cross number one except for a very slight increase of the red tinge with age. This cross is a reciprocal of cross number two.

Cross Number Thirteen.

L.h.z.♀ X L.h.(b)♂

This cross was made for the purpose of studying flower color inheritance. It is a reciprocal of cross number eight. The flowers of all four plants were alike. They showed the same markings as those of cross number eight.



While any discussion of the results of the above crosses is necessarily preliminary, and many points are here passed over that may be of prime importance; it is believed that Mendel's law of inheritance will apply. In each of the twenty four plants of the six crosses described above, the resulting color of the flowers was blue.

The following hypothesis is offered as accounting for the results obtained: The predominant flower color of the different varieties of *Lupinus hirsutus* L. may be represented by two Mendelian factors which are allelomorphic to their absence in each case.

Y. a factor representing a red chromogen.

X. a factor representing an oxidizing agent which acts upon Y to form blue.

Upon this assumption we may represent the constitution of the flowers having the different colors above referred to as follows:

YY xx = Red  
YY XX = Blue  
yy XX = White

In the case of the red flower, the chromogen represented by Y is present but the oxidizing agent which acts upon it is absent and of course the flower is red.

Blue is the color of some flowers because of the presence of both the chromogen and the oxidizing agent.

White is accounted for by assuming that while the oxidizing agent X, is present the chromogen, Y, upon which it acts is absent- hence the pure white color.

This hypothesis will account for the results obtained, though, as stated above, modification or abandonment of it may be necessary when a careful and a further study of the F2 generation of plants is made.