EXPOSURE OF NATURAL GENETIC VARIABILITY UNDERLYING THE PENTAMEROUS COROLLA CONSTANCY IN *LINANTHUS ANDROSACEUS* SSP. *ANDROSACEUS*[1,2]

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L INANTHUS is a member of the Phlox family, Polemoniaceae, and is an organism highly suited for evolutionary, ecological, and developmental genetic studies. HUETHER (1969) described some of its advantages, as well as observations which have been carried out on a large number of natural populations of several Linanthus species. These data concern the percentage of constancy of the pentamerous corolla lobe number in each population, and indicate that this five-lobed condition may be a canalized character in the original sense of WADDINGTON (1940a, 1940b, 1942). WADDINGTON (1961) has suggested that it is fairly obvious that the development of any particular phenotypic character is to some extent modifiable, and to some extent resistant to modification by changes either in the genotype or in the environment. How modifiable will depend upon the importance of the characters to the general fitness of the individual, i.e., there will be selective advantage in building extensive developmental systems for those factors where invariancy is requisite to survival. Experiments by several workers (RENDEL and SHELDON 1960; WADDINGTON 1960; RENDEL, SHELDON and FINLAY 1966; KINDRED 1965) have shown that positive responses to selection for increased canalization can be obtained. Since it is easy to visualize a strong selective advantage in constancy of reproductive structures, corolla lobe number should be among the most invariant of plant characters. Indeed, phenotypic constancy of the five lobes per corolla to the extent of approximately 98 percent was found over diverse habitats, yet as the limits of ecological tolerance were reached, a breakdown in constancy occurred (HUETHER 1969). This was assumed to be due to changes in the developmental processes caused by extreme naturally occurring environmental conditions. The hypothesis that stress environments are the causes of these changes can be tested.

These studies in natural populations of Linanthus have concerned only the phenotype and not the genotype. As canalization of development has been advanced as the cause of the invariance of the pentamerous corolla lobe, a question of importance is what comprises the underlying genetic system. Is it a well buffered homozygous (and homogeneous) system, or one which has considerable

genetic variability? WADDINGTON (1955, 1957, 1961) has suggested that for such phenotypically constant characters, a certain amount of genetic variation, as well as environmental variation can exist, but its effects will be suppressed. Thus, using the normal methods available for exposing genetic variation should provide information to distinguish which of these alternative genetic systems is responsible for the constancy of corolla lobe number.

This paper presents experimental results of two methods used to test whether or not genetic variation exists in natural populations of Linanthus for corolla lobe numbers. The first experiment actually involved a confounding of two methods of exposing variation, as both selection and inbreeding were used simultaneously. Unselected inbred controls and later crossing of the selection lines helped to separate the effects of these treatments, however. The results of five generations of selection clearly point to a large reservoir of hidden genetic variability. Support for these findings also comes from the second method used, that of exposing genetic variation through environmental stresses. Furthermore, this method upheld the hypothesis that extreme environmental conditions were responsible for the increase in abnormal flowers observed in nature. A final method involving transgressive segregation in the F_2 from interpopulational crosses was also employed, but will be presented in a later paper.

MATERIALS AND METHODS

Selection experiment for high and low corolla lobe number: The seeds used for the selection experiment came from a large group of plants taken at random from the S_6 population (see HUETHER 1969) of *L. androsaceus* ssp. *androsaceus* in the spring of 1963 and 1964. This population is located on the Scott Ranch in the Inner Coast Ranges of Yolo County, California. The seeds were bulked, and considered to represent a genetic base comparable to that of the population itself.

Seeds were germinated in vermiculite at 8.9°C in complete darkness for 10 days and then transferred to the greenhouse, where after approximately three weeks they were transplanted to soil and arranged in a completely random experimental design. These procedures were the same for each generation of selection, but the greenhouse conditions varied as generations were grown during the entire year. Control plants from the S_6 bulk seed were grown each generation to determine the effects of these varying greenhouse conditions.

In general, the method of selection is best represented as the usual selection during inbreeding used in producing inbred lines. Self-fertilization was used throughout the study, with the exception of some crossing of lines during the 4th generation. Since the mating system in the 1st generation is not known for certain, the 5th generation material is considered to have been inbred for only 3 generations. Selfing was used principally because of convenience, although it does have other advantages as pointed out by ALLARD (1960), one being that it is especially effective in fixing genes governing characters with low heritability. The coefficient of selection varied between generations due to lack of seeds on some of the selected plants. This was due to several causes, principally because of a high degree of self-incompatibility in some of the plants, but also because of some sterility, due to inbreeding effects and/or adverse by-products of selection.

Experiment 1 on environmental effects. The material used came from bulk seed collected at random in the spring of 1965 from the T_1 population of *L. androsaceus* ssp. *androsaceus*. This is a population in the main East-West transect through the North Coast Ranges of Solano County, California (see HUETHER 1969). Of these seeds, 1310 were planted one month after collection in peat pots of vermiculite (two in a pot), and germinated in two Percival growth chambers at 8.9°C. After most of them had germinated, the plants were thinned to a total of 648. Half of

them were given 10 hours per day of light during a 12 day period of germination and growth immediately after planting; the other half received no light during these 12 days. At the end of this period, all plants were given 10 hours per day of light and 12 hour temperatures of 7.2°C and 19.3°C for 14 days to promote good vegetative growth. Afterward, the plants from each treatment during germination (with light or without light) were randomly divided and put in one of two growth chambers. Thus, of the 648 plants, 324 were in each growth chamber, half of these each having a different germination treatment. One growth chamber continued to give the environment of 12 hours light and temperatures of 7.2–19.3°C (hereafter called the lush environment), while the other growth chamber gave an environment of 18 hours per day of light, and 12 hour temperatures of 19.3° and 32.2°C (hereafter called the stress environment).

Eight days after the difference between the environments was established (35 days from the date of planting), one final treatment was included. Half of the plants in each growth chamber (one-fourth of each treatment during germination) were randomly taken and their main stems removed slightly above the cotyledons. The plants within each growth chamber were then arranged according to a completely random experimental design. These three comparisons (light versus no light, lush versus stress environment, and cut versus uncut stems) represent a hierarchal, or a within-within classification.

An effect of cutting off the main stem is that the data on these plants will all come from lateral branches produced in the axils of the cotyledons. Thus this effect may be confounded with the treatment of cutting, since it is not unreasonable that lateral branches *per se* may produce differences in the number of abnormal corollas. To counter this problem, all flowers were scored as to their positions on the plant, so that in the uncut plants, comparisons could be made between lateral and terminal flowers.

Knowing the effect of germination without lights on the percentage of abnormal flowers produced was felt desirable, since the selection lines were grown in this manner due to the facilities available during the early part of the project. The results were straightforward and showed a 2% increase in abnormal flowers (those deviating from the normal number of five petals) in those plants germinated with no lights compared to those with lights. This was considered reasonable, as darkness during germination would act as a further stress environment on the plants, causing the increased number of abnormal flowers. As these results are not germane to the major purpose of this experiment, and showed a small difference when compared to the other treatments, the data for the lights versus no lights treatment have been pooled in presenting the results.

Experiment 2 on environmental effects: selection lines grown in different environments: The same material that comprised the fifth generation of selection in the greenhouse was used for this experiment. The control plants were again from the bulk seed collection in the S_6 natural population. That this is not the same natural population from which seed was obtained for experiment 1 on environmental effects should be pointed out. Selection lines included were: two selection up lines crossed in the 4th generation, five selection up selfed lines, and eight selection down selfed lines. Selfing in all the material had continued for three generations. Seeds of these lines were planted at one time for all three environments (greenhouse, stress, and lush) and the plants randomly divided into three groups after a 12 day period of germination and growth.

The procedures followed were the same as those for the previous experiment, with the following exceptions. The light versus no light comparison was not included as before, all plants having just the no light period of 12 days after germination in order to be comparable to the previous selection studies. Further, no cutting of any of the plants was carried out, and therefore no data were taken concerning position of the flower on the plant. Lastly, a slight temperature change was made for each environment. This was to increase the lush environment from 12 hours each of 7.2°C and 19.3°C to 10.0°C and 21.1°C, while decreasing the stress environment temperatures from 19.3°C and 32.2°C to 15.6°C and 29.4°C. In the former case, this was done to speed up flowering, while in the latter case the objective was to make the environment slightly less severe, in order that seed might be more easily obtained.

One result of this decrease of temperature in the stress environment was that considerably more flowers were produced on each plant, the average being 42.5 flowers per plant compared

to the previous stress environment average of 15.7 flowers per plant. This is a valid comparison, since, if anything, the inbred selection lines should produce fewer flowers than plants from nature (comparing just the controls, the average was 43.2), and thus demonstrates in a rather persuasive manner the impact of a small temperature change on the physiology of a typical annual plant. Because of this increase in flower production, a limit of 50 flowers per plant in the stress environment was scored as to their corolla lobe number (making the average of 42.5 flowers per plant an underestimate·of a plant's potential). For the lush environment, 25 flowers per plant were recorded for all the plants, with approximately 25% of each treatment being kept at random, so the counts on these plants could be extended up to 50. All data were taken in consecutive flower groups of five, so that comparisons can be made using comparable flower groups, and thereby alleviate possible doubts involving comparisons with different totals.

RESULTS AND DISCUSSION

Selection experiment for high and low corolla lobe number. (1). *General.* Figure 1 presents the results of the first five generations of selection for increase and decrease in petal numbers, and controls for each generation. The graph clearly shows that selection for increased number of corolla lobes has been substantially more effective than that for low numbers. Of the abnormals (those flowers deviating from five lobes) in the controls, the number of flowers with more than five lobes was greater than that of flowers with less than five. These results are interesting in view of the observations from nature presented by HUETHER (1969), where the ratio of abnormals < 5 over those > 5 was about 2.5. The conclusion is that the normal environment of nature causes genes for corollas with few lobes to have a higher expressivity, whereas the abnormal environment of the greenhouse causes genes for corollas with many lobes to have a higher expressivity. This is probably why selection up has been so much more effective. The decrease in this ratio to about 1.5 in natural populations of *L. a.* ssp. *androsaceus* when the limits of ecological tolerance were approached may indicate that the more extreme are the environmental conditions, the more tendency there is for higher numbered abnormals to be produced. Further data to be presented later will add more information on this point. The selection up plants were generally as vigorous as the controls, but those of the selection down lines were in general more depauperate.

While the results of the selection studies to date are clear, one complicating factor of between generation comparisons must be considered. The number of flowers counted per plant was different for each of the generations; in the second generation all the flowers were counted giving an average of 52 flowers on each plant. The next three generations there were 10, 50, and 25 flowers per plant counted, respectively. The significance of this will become clear shortly, but in each generation except the second, the first ten flowers on each plant were recorded and these totals can be compared. When these values are used (only the 4th and 5th generations are affected), selection up for the 4th generation is raised to 29%, while the 5th generation is about 5% higher. This simply straightens out the line and gives it a slightly greater slope. Selection down would stay essentially the same except to drop the 4th generation from 9.1 to 7.6%, also straightening out the line. Again, only the fourth generation changes in the
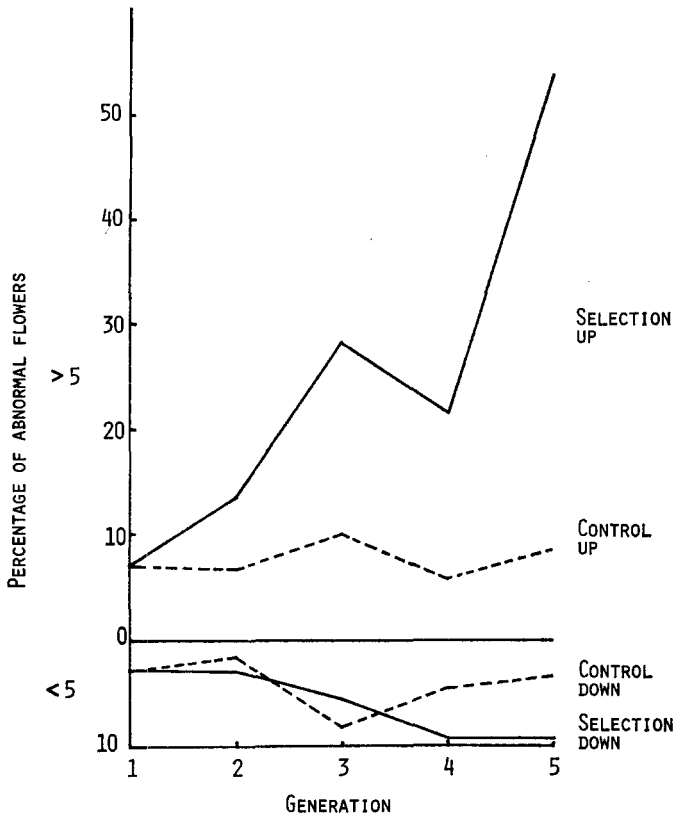
FIGURE 1.—Response to selection over five generations for increase and decrease in number of corolla lobes. The data are given in percentages of abnormal flowers, calculated by using the number of abnormals produced only on the selected side of five. The average number of abnormals in all the controls was used as generation one. The two dotted lines represent above and below five percentages of abnormals in the controls. The solid lines are the results of selection up and down.

controls, control-up moving from 5.9 to 10.0% and control-down from 4.4 to 2.2%. This tends to level out the former line while changing the latter only slightly.

Thus, either way the selection data and controls are presented, essentially the same result is obtained. If anything, presenting the data from only the first ten flowers in the last two generations makes the fit neater, but since these same values were not available in the 2nd generation, presenting the complete totals seemed best. Possibly it should be made clear that within each generation all treatments were scored by the same procedure; i.e., the same number of flowers per plant were counted. The variation in the controls between generations, besides being due to the different scoring procedures, also reflects the difference in environments under which each generation was grown, as well as random error. Plants were grown year-round in the greenhouse so that rather large differences in day-length, light intensity, temperature etc. were encountered. Some regula-

tion of these factors was possible through heating and cooling systems, whitewash, and artificial illumination. However, the majority of the variation should be attributed to the differences in the number of flowers scored.

Within plant variability—4th and 5th generations of selection. Several photographs of the 5th generation selection lines are presented to show the persistent within plant variability found in essentially all the plants. Figure 2 and 3 show individual flowers from 18 of the 21 plants in the best selection up line. Each column of five flowers represents one of the selection up plants. These photographs demonstrate, rather strikingly, two features of the selection results after three generations of selfing; variability within plants and between plants within a line is still present, but the former is less than the latter. If this comparison is continued to the next higher level, that of different lines receiving the same treatment, variability would be greater yet.

A comparison between treatments is given by Figures 4 and 5 where the former figure is that of 40 flowers of one selection up plant, and the latter figure that of 40 flowers of one selection down plant. Both of these plants are among those showing the least amount of within plant variability, yet the corolla lobe numbers in Figure 4 vary from 6 to 10, and in Figure 5 from 2 to 6. However, this comparison of one of the most extreme plants in each treatment after only five generations of selection convincingly demonstrates the magnitude of genetic variation underlying the pentamerous corolla lobe. It also makes evident the greater percentage of abnormals that has been obtained for higher corolla lobes than for lower lobe numbers. This same conclusion was reached earlier from the percentages of abnormals shown in Figure 1, but Figures 2, 3 and 4 point out that on the high side, the range of variability and the mean number of lobes per corolla have increased as well. Further, the results of the highest set of selection up lines in the 5th generation indicate that the number 10 may represent a second zone of canalization, since beyond the normal 5 petals, 10 is the most frequent class greater than 6. Even so, there are still relatively few selected plants in which the first 50 flowers or more are all abnormal. The first plant of this type in the selection down lines has yet to be obtained.

These last four photographs do not indicate anything about the extent of abnormality in early flowers versus late flowers on an individual plant, as the flowers shown were arranged in an arbitrary fashion, and did not represent the entire flower production of any plant. However, differences within plants through time have already been mentioned in respect to the production of abnormal flowers. Data were taken in groups of ten during the 4th generation and in groups of five during the 5th generation such that the first ten flowers, 2nd ten, etc., and first five flowers, 2nd five, etc., could be distinguished in each generation. The results are given in Tables 1 and 2 for the 4th and 5th generation, respectively. Each of the treatments and control in Table 1 show a definite increase in the percent abnormal < 5 as the plants mature and a corresponding increase in percentage > 5. This is particularly clear if the 1–10 flower group is compared to the 41–50 flower group for each column. It appears that the first ten flowers more than any others have a greater tendency to produce more than five lobes,
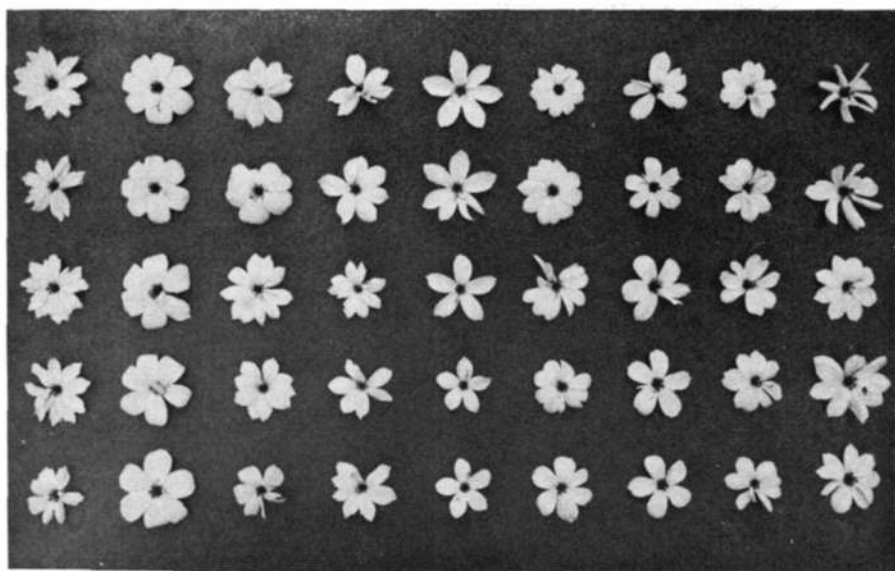
FIGURE 2.—Flowers from nine of the plants in the best selection up line. Each column of five flowers represents one plant. Variability both within plants and between plants within a line is demonstrated.

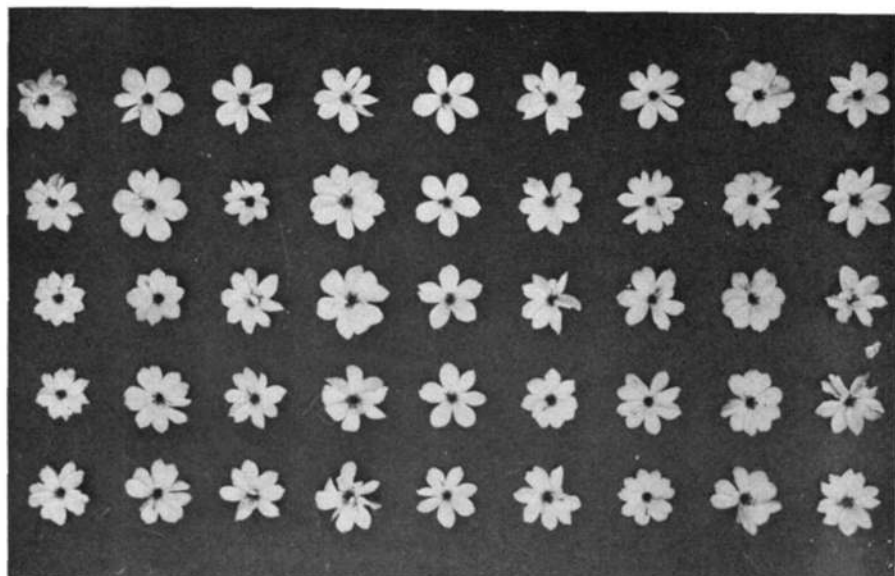FIGURE 3.—Flowers from nine additional plants in the best selection up line. Each column of five flowers also represents one plant.
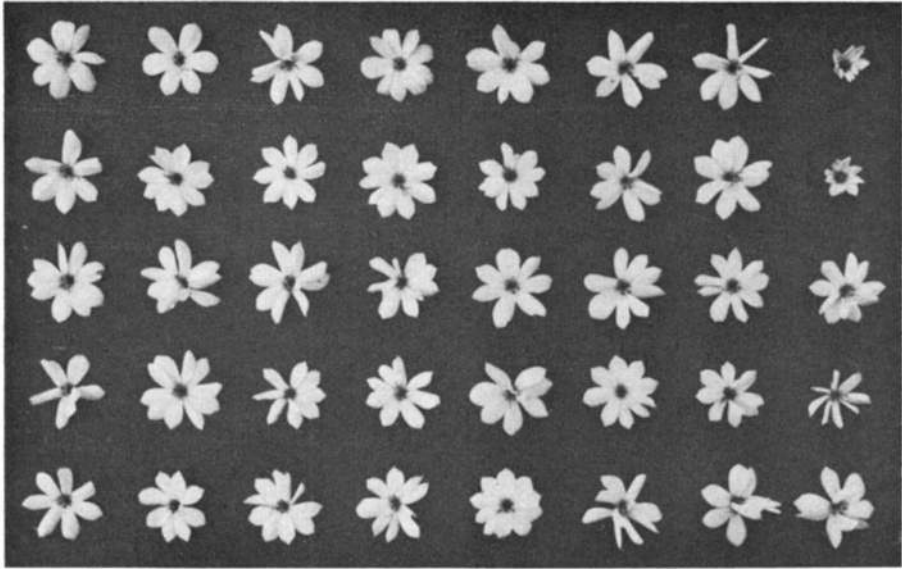
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FIGURE 4.—Forty-five flowers from one plant in a selection up line. Within plant corolla lobe variability is from 6 to 10.
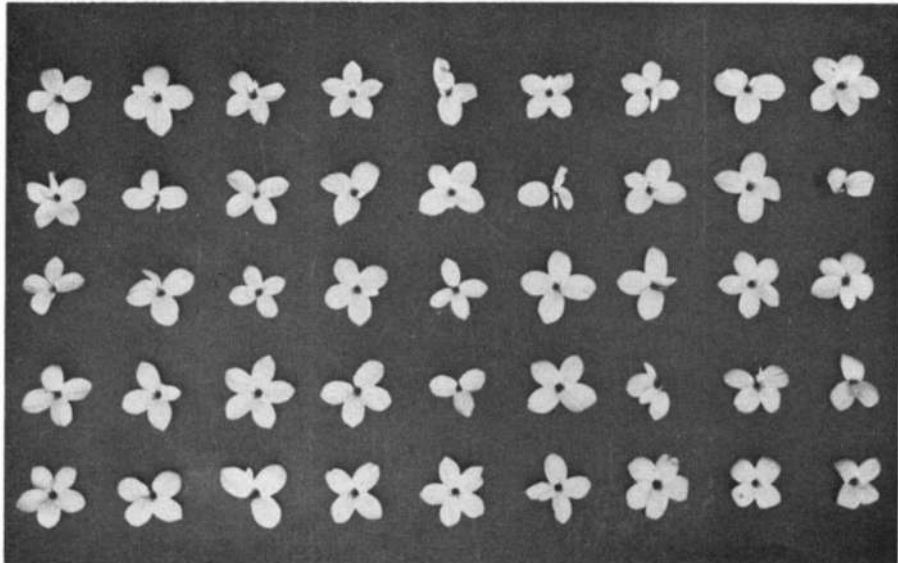

FIGURE 5.—Forty-five flowers from one plant in a selection down line. Within plant corolla lobe variability is from 2 to 6.

TABLE 1

Results of the 4th generation of selection for high and low corolla lobe numbers showing within plant variability

Flowers in each group	Percent abnormal		
	Control	Selection up	Selection down
1–10			
< 5	2.22	1.65	7.63
> 5	9.95	30.40	5.97
Total	12.17	32.05	13.60
11–20			
< 5	3.81	1.51	7.79
> 5	3.81	19.86	3.36
Total	7.62	21.37	11.15
21–30			
< 5	4.29	2.12	8.63
> 5	4.95	20.16	4.04
Total	9.24	22.28	12.67
31–40			
< 5	6.35	3.47	11.36
> 5	5.01	18.63	2.25
Total	11.36	22.10	13.61
41–50			
< 5	6.26	5.11	12.32
> 5	5.31	17.65	3.22
Total	11.57	22.76	15.54
0–50			
< 5	4.40	2.55	9.15
> 5	5.92	22.08	3.98
Grand total	10.32	24.63	13.13
Grand total of flowers counted	4496	8839	4799

Flowers were counted consecutively in groups of ten for each plant in a treatment. Percentage of abnormal flowers for each group are given with a further breakdown into those < 5 and > 5. Those plants not producing 50 flowers were scored until they died.

since the control and the two selection up columns drop substantially in the 11–20 flowers group (10.0 to 3.8%, 33.0 to 20.4%, and 25.3 to 18.9%, respectively), but then stay relatively the same. The increase in abnormals < 5 generally offsets the decrease in abnormals > 5 in both the control and selection down, so that the total percent abnormal for each flower group is roughly the same. However, this is true only for selection up after the initial drop of abnormals > 5 from the 1–10 flower group. Selection up is seen to be more effective than selection down in every flower group, even in the late flowers where the

control is producing more abnormals < 5 than > 5. While 50 flowers in groups of 10 were counted in the 4th generation, only 25 flowers in groups of 5 were scored in the 5th generation. Even so, the 5th generation results shown in Table 2 are in good agreement with the trends of the previous generation. A decrease in abnormals > 5 was detected in later flowers for each treatment and the control. The increase in abnormals < 5 is lacking in each column except the controls, but is not expected due to the count ending with 25 flowers. This expectation is based on the significant increase of abnormals < 5, in the previous generation, coming in the 30–50 flower groups.

In Table 2, high selection is listed as two categories; the distinction is made on the 4th generation mating system. The first set of data are from plants selfed

TABLE 2

Similar to Table 1 but for the 5th generation of selection

Flowers in each group	Percent abnormal			
	Control	Selection up	Selection up (crosses)	Selection down
1–5				
< 5	3.17	1.10	0.57	9.85
> 5	10.17	54.52	64.00	9.85
Total	13.33	55.62	64.57	19.70
6–10				
< 5	3.17	0.83	0.29	8.66
> 5	11.17	47.50	62.18	7.76
Total	14.33	48.33	62.47	16.42
11–15				
< 5	2.33	1.11	0.29	9.25
> 5	8.17	44.60	62.57	5.67
Total	10.50	45.71	62.86	14.92
16–20				
< 5	4.17	1.11	0.29	8.06
> 5	7.50	45.96	60.28	3.88
Total	11.67	47.07	60.57	11.94
21–25				
< 5	3.86	0.28	0.29	9.39
> 5	6.89	41.27	58.57	1.82
Total	10.76	41.55	58.86	11.21
0–25				
< 5	3.34	0.89	0.34	9.04
> 5	8.78	46.84	61.52	5.81
Grand total	12.12	47.73	61.86	14.85
Grand total of flowers counted	2995	1804	1749	1670

Here flowers were counted consecutively in groups of five rather than ten. The results of interline crossing of superior plants in the selection up lines during the 4th generation are listed separately.

as usual; the second set is from crosses made between superior plants in different selection up lines. These crosses were made to determine to what extent the extreme form of inbreeding used was responsible for advance under selection. The results of 61.8% abnormal in the crosses compared to 47.7% under continued selfing show that no restoration of normality was obtained by an increase in heterozygosity. The fact that the crosses gave rise to more abnormals than the selfs probably reflects the higher percentage of abnormals in the plants used. No definite conclusions can be reached concerning homozygosity versus heterozygosity at the present time, however, since the selfed selection lines are known to be inbred for certain in only the three previous generations. Some data from unselected inbred plants have been collected as an alternate approach to studying the effects of increased homozygosity. These are also in the S_3 generation and have shown significantly higher percentages of abnormals compared to the controls in both the S_2 and S_3 (13.07% in the S_2 versus 10.32% in the control, and 18.20% in the S_3 versus 12.12% in the control). Beyond this, however, the inbreeding effect is unclear, as the significant differences in the S_2 arose because of a greater percentage of abnormals < 5, whereas in the S_3 the difference was due to those > 5.

In the controls of both generations, the ratio of percent abnormals < 5 over those > 5 starts out at about 0.25 in the early flowers and increases to about 1.2 in the later flowers. Since most of the plants produce more than 50 flowers, a further increase in this ratio could reasonably be expected in the latest flowers. Previously, this ratio in natural populations was seen to be approximately 2.5 during the peak of flowering and drop to about 1.5 as the limits of ecological tolerance were approached; the conclusion being that the more extreme environment in nature causes production of higher numbered abnormal flowers. The opposite conclusion is reached on the basis of the greenhouse results, assuming there must be a greater physiological stress on the plants as the end of flowering is approached. The distinction should be made clear, however, that the greenhouse results are due to within plant variability under the same environmental conditions, while the natural population results are due to between plant variability presumably caused by the known different environmental conditions. In the former case, the increase in the ratio is on the same plant and due to an internal cause; in the latter case, the decrease in ratio is a comparison of plants in different environments, and due to an external cause. Another factor causing these differences could be the great diversity between greenhouse and natural conditions.

In conclusion, the striking feature of these data when listed by flower groups is the consistent decrease of abnormal flowers > 5 in the selection lines and the controls with increasing age of the plants. In the later flowers particularly, there is a concomitant increase in the number of abnormals < 5, offsetting this decrease to varying degrees.

Experiment 1 on environmental effects. (1) *General.* The purpose of this experiment was two-fold: (i) to ascertain the extent of genetic variability underlying the pentamerous corolla lobe in nature by using the method of environmental

stress to expose genetic variation, and (ii) to test the hypothesis presented by HUETHER (1969) that the environmental stresses of long-day-length and high temperatures were responsible for the increased number of abnormals observed in nature toward the end of the flowering season, and on plants decapitated by ground squirrels. This amounts to an attempt to simulate peak and late flowering conditions in natural populations, although they are more extreme than would be encountered in nature. The treatment of cutting off the main stem of some plants was carried out to simulate the activity of the ground squirrel in some of the natural populations.

The results pertaining to the first objective are summarized by genotypes in the lush and stress environments in Table 3. The fact that 33.6% of the plants in the lush environment produced at least one abnormal compared to 82.8% under the stress conditions points out the substantial part the environment can play, but contains no information about genetic variability. The significant differences between genotypes are shown in the totals of each environment, but more strikingly in those of the stress. These stress totals show a wide distribution of the plants in regard to the number of abnormals they produced, pointing to real genetic differences in this material taken at random from a natural population. The totals of the lush environment also show a few genotypes producing a large number of abnormals compared to the majority of the plants. Genetic variability here is seen in that less than 10% of the plants gave more than 60% of the abnormal corollas. In both environments these data are clearly very different from a normal type of distribution expected on the basis of random distribution of abnormal flowers throughout all of the genotypes. Thus, the significant point of the data from two constant environments is the substantial variability

TABLE 3

Results of experiment 1 on environmental effects listed by genotypes

Treatment	Number of abnormal flowers per genotype										Total abnormal genotypes	Total genotypes
	0	1	2	3	4	5	6–10	11–15	16–20	>20		
	lush environment											
uncut	97	22	17	12	5	2	3	.	2	.	63	160
cut	114	23	11	5	1	1	2	.	1	.	44	158
Totals	211	45	28	17	6	3	5	.	3	.	107	318
	Abnormal plants = 107/318 = 33.6%											
	< 10% of the genotypes gave > 60% of the abnormals.											
	stress environment											
uncut	24	26	30	17	11	10	29	8	1	1	134	158
cut	29	25	24	21	17	9	17	8	.	.	121	150
Totals	53	51	54	38	28	19	46	16	1	1	255	308
	Abnormal plants = 255/308 = 82.8%											

A comparison is made between the lush and stress environments as to the number of abnormal corolla lobes produced per genotype for the two environments. Whether or not the plants were cut is listed for both environments. Over twice as many plants in the stress environment had at least one abnormal as compared to the lush environment.

between genotypes revealed by these unnatural conditions. Statistically, there is no difference between cut and uncut plants for either environment in the number of genotypes producing at least one abnormal.

The impact of the environment is, if anything, diminished in this table, since the mean number of flowers per plant was 24.2 in the lush environment compared to 15.7 in the stress. Thus, on the average, each plant under lush conditions had almost nine more flowers in which to produce an abnormal, yet there were still four times as many plants with all normal flowers in the lush compared to the stress environment. One point to be brought up about these data is that the number of flowers counted per plant did vary slightly depending on when the plant died in the stress environment, and also how rapidly it flowered in the lush conditions. However, in reviewing the individual plant data, this detracts very little from the total results, since most plants had close to the same number of flowers scored.

For answering the second question of the experiment, the results are tabulated in a different form in Table 4. The hypothesis tested is that both late season flowering and ground squirrel decapitation of the plants in nature caused the increase in abnormal flowers through the environmental stresses of long day-length and high temperatures. This says that not only should the lush versus stress environment comparison produce a significant difference in abnormals, but that no difference should be obtained between cut and uncut plants in either environment. The data in Table 4 showing percentages of abnormal flowers for each of the four treatments make it clear that the increase of abnormals in the stress environment is highly significantly different from the lush environment (the contingency table x^2 for the comparison of the lush versus stress environ-

TABLE 4

Results of experiment 1 on environmental effects listed by percentages of abnormal flowers obtained

Treatment		Flower totals	Percent abnormal			
			< 5	> 5	Total	
lush	cut	3615	0.77	2.02	2.79	
lush	uncut	4087	1.66	2.69	4.35	1.56% abnormal due to not cutting.
stress	cut	2502	9.23	9.23	18.46	15.67% abnormal due to stress environment.
stress	uncut	2333	11.44	13.67	25.11	5.09% abnormal due to stress and uncut interaction.
stress	. . . .	4835	10.30	11.38	21.68	
lush	. . . .	7702	1.25	2.38	3.63	
. . . .	uncut	6420	5.22	6.68	11.90	
. . . .	cut	6117	4.25	4.97	9.22	

Each of the four treatments is given with percent abnormals < 5 and > 5, flower totals, and the percentage of increase in abnormals for which they, and their interactions, are responsible. Summation percentages for each classification are also given.

ment in the uncut plants is 411.8, and in the cut plants is 144.8 compared to the tabulated x^2 at the .01 level of 6.63). Summing over the cutting treatments within each environment, the comparison is 21.68% abnormal flowers to 3.63%, a sixfold increase. Comparing cut versus uncut, in each environment there are *fewer* abnormals in the cut plants than in the uncut plants. In fact, this comparison also gives a highly significant difference (the x^2 value in the lush environment is 12.99 and in the stress is 31.18). However, in order to reject the hypothesis that decapitation of the plants played no role in causing the increase of abnormals in nature, *more* abnormals in the cut plants should have been obtained. Thus, the hypothesis is supported in each respect by these data.

Table 4 also shows the percentage of increase in abnormals for which each of the treatments, and their interactions, are responsible. While the effect of not cutting the plants has been pointed out to be significantly different than the cutting effect, this arrangement emphasizes the difference to be only 1.56%. Most of the difference between these two treatments in the stress environment is seen to be due to a second order interaction between the environment and the effect of not cutting the plants. To understand the reason for the 1.56% increase, the fact that two further treatments of lights versus no lights were used during germination must be recalled. When the data from the lush environment are broken down further including these treatments with the cutting treatments, a second order interaction between the no lights and uncut treatments is seen to account for 1.38% of the 1.56 increase in abnormals flowers. This shows that there is only a .18% increase accounted for by not cutting the main stem of the plants, exclusive of interactions, and hence that there is little difference between the main effects of cutting and not cutting plants when only these two treatments are considered. As this was the only important effect that the lights versus no lights treatments had on the data of this experiment, it was considered the results would be clearest if these treatments were not included in the tables.

(2). *Within plant variability.* (i). *Through time.* Table 5 presents the data of this experiment in consecutive flower groups of ten as was done in some of the later generations of the selection experiment. These data show that the substantial differences in totals for abnormal percentage between lush and stress environments are maintained in each of the flower groups. Furthermore, the numbers of flowers counted are such that their ratio between flower groups is roughly the same comparing lush *versus* stress in both the cut and uncut treatments. This points out that even substantial within plant variability picked up by differences in the number of flowers counted in each environment could not affect the total lush and stress comparison to any large extent.

This table adds further information to that already obtained concerning early *versus* later flowering and its effect on producing abnormal flowers. These data show the same trend as found earlier in having a higher percentage of abnormals < 5 as the plants mature, but the increase in three of the four classifications comes earlier in flowering. The lush environment for both cut and uncut plants also produced an increase in abnormals > 5, which is the opposite of the stress environment, as well as that observed in the selection studies. Thus these data

TABLE 5

Results of experiment 1 on environmental effects listed by consecutive flower groups of ten

Flower group	Lush				Stress			
		Percent abnormal				Percent abnormal		
	Flowers	< 5	> 5	Total	Flowers	< 5	> 5	Total
				cut				
0–10	1567	0.70	1.66	2.36	1307	6.58	11.09	17.67
11–20	1199	0.58	2.08	2.64	792	11.99	7.58	19.57
21–30	609	0.49	2.30	2.79	304	11.84	6.91	18.75
31–65	240	2.92	3.33	6.25	99	14.14	5.05	19.19
				uncut				
0–10	1591	0.19	2.07	2.26	1272	8.02	13.13	21.15
11–20	1369	1.31	2.70	4.01	659	15.02	15.17	30.19
21–30	727	3.44	2.61	6.05	255	16.47	14.51	30.98
31–65	400	5.50	5.25	10.75	147	16.33	10.20	26.53

The total number of flowers, and percent abnormal < 5, > 5, and totals are given for each flower group in the four treatments.

show that extreme differences in environments can be expected to cause differences in the variability of each plant through time, as well as in total number of abnormals produced. Even so, the trend of the ratio of percent abnormals < 5 over those > 5 to start out low in the early flowers and to increase in the later flowers remains consistent.

(ii). *Position of the flower on the plant.* At the outset of this experiment, there was some concern over the possibility that any differences found between the cut versus uncut comparison would be confounded through the flowers having a different position on the plant. If lateral branches *per se* cause differences in the number of abnormal flowers produced, compared to the main stem inflorescence, then part or all of the differences found between decapitated and normal plants in nature may be explained on this basis, as normal plants seldom have lateral branches under natural conditions. While this difficulty did not materialize due to the close agreement between the results in both treatments, the data collected are interesting in themselves, and are therefore presented in Table 6. Each flower was scored as being in the terminal inflorescence of the main stem in the uncut plants, or on one of the several lateral branches. For the cut plants, lateral branches soon appeared, not only from the axils of the two cotyledons, but on these branches themselves. Hence, flowers on these plants were also recorded in two categories, being either in the terminal inflorescence of the main lateral branches (listed in the table as terminal), or else as one of the laterals off of these branches (listed in the table as lateral).

The important comparison is between the percentages of abnormals among terminal and lateral flowers in the uncut plants. For both the lush and stress environments, there is a highly significant difference between the number of abnormal flowers produced at these positions (6.6 *versus* 2.9% for the lush environment, and 31.4 *versus* 22.6% for the stress). In each environment, this

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TABLE 6

Results of experiment 1 on environmental effects listed by position of the flowers on the plant

Flower position	Lush				Stress			
		Percent abnormal				Percent abnormal		
	Flowers	< 5	> 5	Total	Flowers	< 5	> 5	Total
			cut					
terminal	1945	0.67	1.80	2.47	1637	8.86	9.71	18.57
lateral	482	1.45	1.66	3.11	362	12.71	7.73	20.44
	$\chi^2 = 2.95 < 5.99 = \chi^2_{.05(2)}$				$\chi^2 = 6.37 < 7.81 = \chi^2_{.05(3)}$			
			uncut					
terminal	1903	0.37	2.52	2.89	1410	8.94	13.62	22.56
lateral	1214	3.21	3.38	6.59	625	16.96	14.40	31.36
	$\chi^2 = 43.46^{**} > 9.21 = \chi^2_{.01(2)}$				$\chi^2 = 30.27^{**} > 15.1 = \chi^2_{.01(5)}$			

The percentage of abnormal flowers < 5, > 5, and totals are given for flowers on the terminal inflorescence of the main stem, and those on the lateral branches, for the uncut plants in the lush and stress environments. In the cut plants, those listed as terminal refer to the terminal inflorescence of the lateral branches that came from the axils of the cotyledons; the other flowers came from branches off of these two main laterals.

difference is due principally to an increase in the number of abnormals < 5 occurring in flowers on the lateral branches. Even though this difference in flower position is real, it is not nearly sufficient by itself to account for the 32% of abnormals found in the decapitated plants of nature, since the increase is only 3.7% and 8.8% for each environment, respectively. However, coupled with the stress environment, it duplicates the observations in nature (31.4 *versus* 31.7%, respectively).

An interesting point to consider further is that each environment gave parallel results concerning the large increase in abnormals > 5, and the slight increase in abnormals < 5, on the lateral branches. This does not reflect the changes that the different environments caused in the variability of the plant through time, shown in the flower group comparisons of Table 5. Thus in producing abnormals, the position of the flower overcame the differences in environments to a greater degree than early *versus* late flowering, probably because the position of the flower is not as critical for producing abnormals as the change in physiology of the plant from early to late flowering.

This same parallelism of action of the environments on terminal and lateral flowers is seen in the cut plants. Both environments cause an increase in abnormals < 5 as in the uncut plants, but also a slight drop in abnormals > 5, although none of these changes are significant. The lack of significance is important, because, along with the point in the preceding paragraph, it helps to exclude the possibility that flowers on lateral branches are simply a reflection of late flowering. This relationship may be true to some extent, but these non-significant differences between terminal and lateral flowers in the cut plants, compared to the significant differences in the uncut plants, show that there is not a high correlation between late developing flowers and those on lateral

branches. Support for this also comes from the great deal of similarity in cut and uncut plants in each environment.

The non-significant differences in the cut plants reflect the fact that all of these flowers came from lateral branches. The surprising factor that still remains is that these flowers gave a percentage of abnormals more like the terminal rather than the lateral flowers in the uncut plants, thus accounting for the lower percentage of abnormals in the overall comparison between the cut and uncut treatments. It is reasonable to suppose that when the main stem is taken away, the lateral branches act to replace it, thereby becoming more like it both developmentally and physiologically.

In conclusion, this first experiment on environmental effects has supported the selection experiments in showing, through the use of growth chamber environments, a considerable amount of underlying genetic variation for this canalized character. It has presented data in agreement with the hypothesis that environmental stresses of long day-length and high temperatures were responsible for the increased number of abnormals toward the end of the flowering season in nature, as well as mostly responsible for the increase observed on plants decapitated by ground squirrels. This increase was to some extent found to be due also to the effect of lateral branches *per se*, and the interaction between the two. Within plant variability was in general found to be similar to that reported in the selection studies.

Experiment 2 on environmental effects: selection lines grown in different environments. In view of the success that was obtained using a stress environment to produce a substantial increase in abnormal flowers, learning how the selection lines would react to these conditions was considered desirable. The selection experiments had all been grown in the greenhouse, and while the conditions there varied from generation to generation, the variation was not as great, or as precisely controlled, as that used in the experiment just described. Therefore, the purpose of this experiment was again twofold: (1) to find whether or not the selection lines maintain their level of advance under the lush environmental conditions of growth (which approximate closely the natural environment in regard to percentage of abnormal flowers produced, i.e., 3.63% *versus* 2.52%), (2) to learn how two methods of producing abnormal flowers, *viz.*, selection and an environmental stress, will act when used together. The interaction may be *additive*—the total number of abnormal flowers obtained will be equal to the sum of those induced by each method separately; *antagonistic*—the total number will be less than the number induced separately; or *transgressive*—the total number will be greater than the sum of those induced separately.

The results pertaining to the first objective are presented in Table 7. The data from these same lines when grown under the usual greenhouse conditions are given for comparison, and designated with G.H. All comparisons are based on 25 flowers per plant. What is seen in the table is that both the selfed and crossed selection up lines are considerably lower than the levels they attained in the greenhouse (24.85 and 28.52%, respectively). At the same time, they are considerably higher than the lush environment control, being almost midway be-

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TABLE 7

Results of the selection lines and a control grown under lush conditions

Treatment	Abnormal < 5	Abnormal > 5	Abnormal totals	Total flowers
selection up	0.65	29.43	30.08	768
selection up—G.H.	0.62	54.28	54.91	805
selection up (crosses)	0.11	42.67	42.78	921
selection up (crosses)—G.H.	0.10	71.19	71.29	1024
selection down	8.81	1.87	10.68	1282
selection down—G.H.	9.40	5.83	15.24	1595
Control	0.80	1.46	2.26	1368
Control—G.H.	3.14	9.13	12.27	1720

The percentage of abnormal flowers < 5, > 5, and totals are given for each of the treatments and control. The results of these same lines under greenhouse conditions (marked as G.H.) are given for comparison. All figures are based on 25 flowers/plant.

tween the two extremes in both cases. Therefore, the conclusion is that approximately half of the advance resulting from selection up is lost under an environment approximating nature, but also that half is retained. These results are similar to those obtained by BATEMAN (1959a, 1959b). She employed a temperature treatment of the pupae of Drosophila as the abnormal environment, and after a varied number of generations of selection for several characters found that 12–24% and as high as 46% of the selection advance was retained when the flies were allowed to develop under normal conditions. The selection down results under the lush environmental conditions are better than selection up, in that practically all the abnormal flowers < 5 remain (8.8 *versus* 9.4% in the greenhouse), while the number of abnormals > 5 drops close to the level of the lush environment control (1.87 *versus* 1.46%). In this case the advance obtained in the greenhouse appears fixed for other environments as well, although it is not as high as that in the selection up lines.

Results pertaining to the second objective of this experiment are given in Table 8. The greenhouse results are again included for comparison. For both mating systems in the selection up lines, the stress environment has increased

TABLE 8

The same as Table 7, except grown under stress environmental conditions

Treatment	Abnormal < 5	Abnormal > 5	Abnormal totals	Total flowers
selection up	1.71	57.06	58.77	878
selection up—G.H.	0.62	54.28	54.91	805
selection up (crosses)	0.00	81.99	81.99	722
selection up (crosses)—G.H.	0.10	71.19	71.29	1024
selection down	12.44	8.68	21.12	1302
selection down—G.H.	9.40	5.83	15.24	1595
Control	2.84	18.80	21.65	1372
Control—G.H.	3.14	9.13	12.27	1720

the level of advance beyond that obtained in the greenhouse. If these two artificial means of causing variation were working on a strictly additive basis, the increase in abnormals should have been by the amount of the difference in the two controls, *viz.* 18.80–9.13%, or 9.67% beyond the greenhouse percentages. The crossed selection up lines attained this advance to within 1%, although the selfed lines fell short by almost 7%. The type of interaction which appears to be involved in each case, however, is of an additive, or partly additive nature, rather than a negative or transgressive type. For the selection down line, the difference in controls is −0.30%, and on this basis, a transgressive interaction is indicated for the number of abnormals < 5, as an increase of 3.04% was obtained. An increase in abnormals > 5 was also obtained, but to a lesser extent than in the controls. These data suggest that the genetic systems being produced by upward *versus* downward selection are not the same, in that they interact in a fundamentally different way with stress environmental conditions.

A comparison between the total percent abnormal in the controls in the lush environment of this experiment with those of experiment 1 on environmental effects shows highly significant differences, but very similar ratios of percent abnormal < 5 over those > 5, while these comparisons in the stress environments show similarity in total percent abnormal, but very different ratios of percent abnormal < 5 over those > 5. However, two treatment effects are confounded, as not only was a slight temperature increase and decrease used in the lush and stress environments, respectively, in the second experiment, but also the seeds came from a different natural population and therefore different gene pools are represented in each experiment. Further experimentation will be needed to show which of these treatments is responsible for the differences obtained.

Since the comparisons made regarding the main objectives of this experiment have all been based on the same number of flowers per plant, no difficulty due to within plant variability is involved. The data were collected in flower groups of five for the first fifty flowers however, so that more information on within plant variability could be obtained. As the results are similar to those previously presented, the actual data have not been included. Only the plants in the lush environment did not conform fully to the characteristic changes found previously, in that the two selection lines under these conditions did not show an increase in abnormals < 5 as the plants matured, nor did the control show the characteristic drop in abnormals > 5. As seen earlier, this drop was also not obtained in the lush environment of experiment 1.

<center>DISCUSSION</center>

This paper has been mainly concerned with showing the broad genetic base underlying the constancy of five corolla lobes in *Linanthus androsaceus* ssp. *androsaceus*. It has been shown that far from genetic homozygosity or homogeneity being the cause of this constancy, substantial genetic variability exists in natural populations. The assumption has been that developmental buffering (canalization) is responsible for maintaining the pentamerous condition. WADDINGTON (1940a, 1940b, 1942) suggested that when it is of advantage to an

organism for its development to follow a certain path, a genotype will evolve which will resist slight abnormalities of the genotype or of the external environment; in his terminology the developmental path, or creode (WADDINGTON 1957) is canalized. The emphasis is on phenotypic invariance of the end product which comes about through a buffering of the developmental path so that it resists change. Regulatory interactions during histogenesis and morphogenesis are considered by WADDINGTON to produce this buffering effect. While more detailed knowledge of these mechanisms remains elusive, there have been many experiments in mice and Drosophila where support for genetic variation underlying constant characters has been found. Environmental stress, often in the form of temperature shock, as well as major gene mutations (*Tabby* mutant in mice and the *scute* mutant in Drosophila) have been employed to expose this underlying genetic variability, which has then been responsive to selection away from the normal trait (see WADDINGTON 1961 for a review of some of the experiments on environmental stimuli; MILKMAN 1960a, 1960b, 1962). In the present investigation, the environmental stress used for the selection experiment was that supplied by the greenhouse conditions, as the percentages of abnormals increased by a factor of four from the average found in nature (2.52 *versus* 10.12%). The growth chamber environments supplied more severe conditions and showed even greater underlying genetic variation upon which further selection could be carried out. This appears to be the first such work carried out in plants, and has allowed greater precision in relating experimental work back to known conditions in natural populations.

In the one natural population (S_6) used for selection, an asymmetry in response to selection in both directions away from five lobes was consistently obtained. FRASER and KINDRED (1960) also found an asymmetrical response when selecting for increased and decreased number of vibrissae in the presence of the *Tabby* allele. In the present work however, a comparison of the ratio of percent abnormal < 5 over those > 5 between the T_1 population in experiment 1 (.85) and the S_6 in experiment 2 (.15) in similar stress environments (difference of 2.8°C) suggests downward selection may be more effective if practiced in other natural populations. Also, the preponderance of abnormals being < 5 in nature led to an earlier conclusion that differences in environments cause different genes to have higher expressivity, which indicates there may be environments similar to those in nature where down selection would be effective.

To summarize all of the data presented on within plant variability, including the selection studies and both environmental effect experiments, 14 of 17 comparisons had a definite drop in abnormals > 5 as the plants aged, and 14 of 17 had an increase in abnormals < 5, but in only 5 of the 17 was there a greater total percent abnormal in the last flower group compared to the first flower group, 4 of these 5 being in experiment 1 on environmental effects where no selection was involved (see Table 5). This means that in 12 of 17 cases, constancy of the pentamerous condition is approached from early to later flowering, presumably as physiological stresses on the plants increase. If this is so, then internal *versus* external stresses have different influences on the degree of corolla lobe constancy.

Finally, the trend of the ratio of percent abnormal < 5 over those > 5 to become larger in the later flowers was consistent in all but two of the 17 comparisons.

A suggestion has been made (STEBBINS 1966) that the number of petals on a flower depends upon the total number of cells produced by the floral meristem which have the differentiation capacity for a petal, divided by the number of meristematic cells needed per petal primordium. This latter factor might be expected to stay relatively constant during the flowering period of any one plant, but the total number of cells produced could vary with time, either through alterations in size of the undifferentiated meristem, or variation in time over which the meristematic cells possess a particular differentiation capacity. From the present data showing a definite decrease in the percentage of higher corolla lobe numbers, it would appear that the total number of cells differentiating into petals was being reduced as the plants mature. Since most of a plant's energies during late flowering are being diverted into the production of seed from earlier flowers, this decrease might be expected. The data in the lush environments showing an increase in abnormals > 5 is in agreement here, since these plants were much stronger and more vigorous, suggesting that the usual late stresses on the plants were not felt under these conditions. Assuming that in late primordia fewer meristematic cells are available, the fact that there is a general increase in abnormals < 5 is also in agreement, as well as the rise in the ratio of percent abnormal < 5 over those > 5. The trend toward fewer abnormals in the later flowers, or increased constancy of five lobes, is explained by the large loss of abnormals > 5.

Experiments reported by ROY (1958a, 1958b, 1959, 1963) and HESLOP-HARRISON (1959) are pertinent to the present investigation, in that they pertain to the same character, even though in species belonging to different plant families. ROY's work on *Nyctanthes arbor-tristis* of the Verbenaceae showed these flowers to be not as well canalized for the modal number of six petals as those of *L. a.* ssp. *androsaceus*, but significant within plant variability was also obtained. The results of these separate studies are difficult to compare closely however, since *N. arbor-tristis* is a perennial compared to *L. a.* ssp. *androsaceus* as an annual, a fact which surely must call for substantial differences in physiological changes during their respective flowering seasons. Also, ROY's measurements were taken in the compound of the Indian Statistical Institute, Calcutta over a seasonal change from late September to the middle of January. HESLOP-HARRISON observed that for plants of *Cannabis sativa* (hemp) grown in day and night temperatures of 22°C, the within-plant variances for petal and stamen number were low (0.0185 and 0.0437, respectively). However, when plants were grown with an eight hour night temperature of 10°C, within plant variance increased to 0.3290 and 0.6601, respectively. These result are comparable to the increase in abnormals in the stress environment effects of experiment 1, and are another striking instance of the effects of severe environmental stress upon developmental regulation.

To summarize the main findings of this paper, Figures 6 and 7 are included. Figure 6 shows, pictorially, the effect of selection and environmental stress on
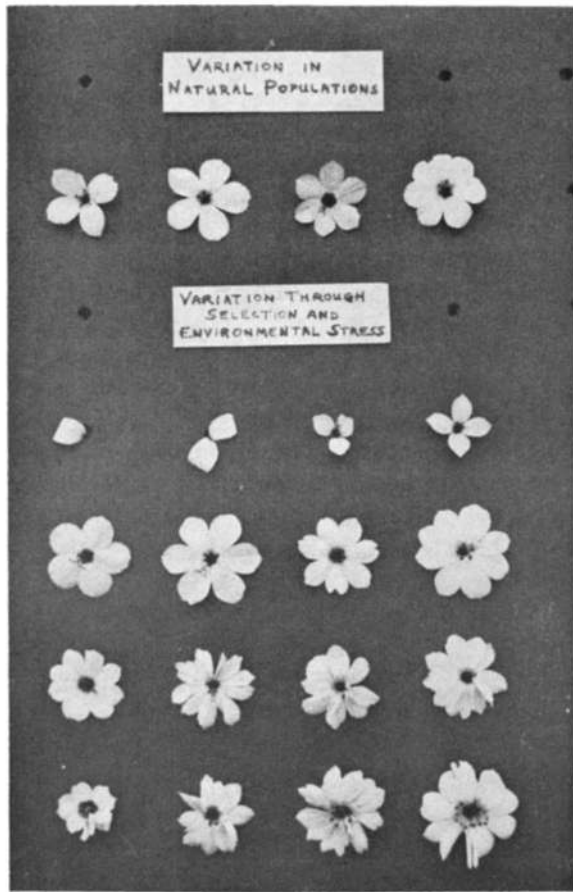
FIGURE 6.—Flowers representing variation observed in natural populations, compared with that obtained through selection and environmental stress experiments. The former is shown with four to seven lobed flowers, while the latter is demonstrated with corollas ranging consecutively from one to sixteen.

the variation in corolla lobe number, as opposed to phenotypic variation in natural populations. A range of from 4 to 7 petals was generally found in nature, with a rare deviant beyond this. However, a petal range from 1 to 16 (occasionally higher) per flower was produced using these two means of exposing genetic variation. Figure 7 presents these results in graphic fashion. The total percentage of abnormal flowers obtained experimentally by each means is given and compared to those obtained in nature.
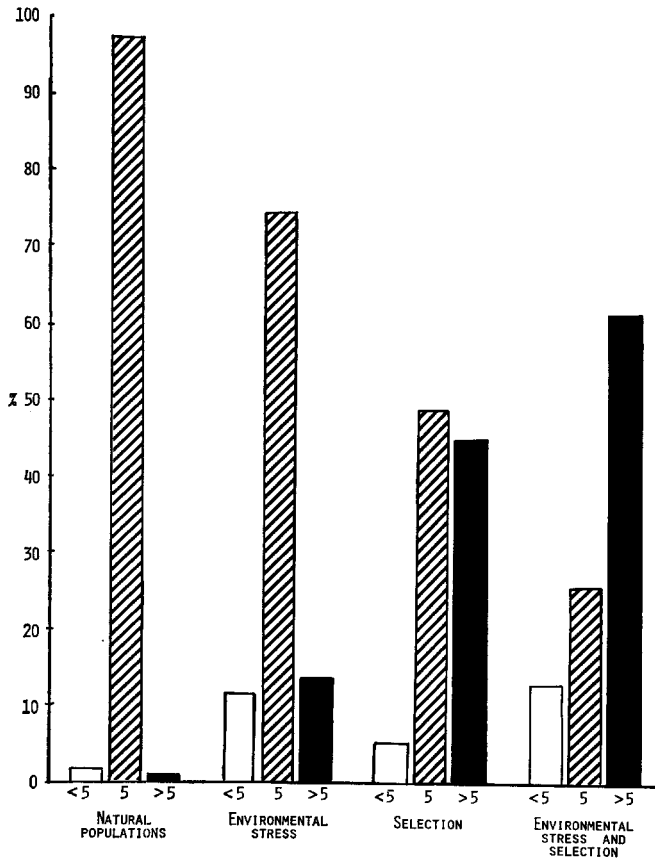
FIGURE 7.—A comparison of percentages of abnormalities in corolla lobe number from four independent investigations.

SUMMARY

In order to test whether the invariance of the five-lobed corolla is due to a well buffered homozygous (and homogeneous) system, or one which has considerable genetic variability, two methods of exposing genetic variation were employed, *viz.*, selection experiments (with inbreeding) and environmental stresses. After five generations of selection for both increase and decrease in the number of lobes per flower, upward selection was found to be much more effective under the experimental conditions of the greenhouse than was downward selection, although both selection schemes showed the existence of considerable genetic variation underlying the pentamerous corolla constancy. Upward selection in particular was enhanced by these environmental conditions, in that the percentage of abnormal flowers < 5 (1.44) and the percentage > 5 (0.57) found in nature (HUETHER 1969) rose to 2.94 and 7.18, respectively, in the controls. Further support for the presence of considerable genetic variation came from growing seeds, collected at random from a natural population, under two sets of

uniform conditions, one having low temperatures and short day-length, and the other high temperatures and long day-length. For instance, under the former conditions of growth, less than 10% of the genotypes produced more than 60% of the abnormal flowers.—Observations on within plant variability of corolla lobe numbers were also made. The striking feature of these data when listed by consecutive flower groups of five or ten was the consistent decrease in abnormals > 5 with increasing age of the plants. In the later flowers particularly, there was a concomitant increase in the number of abnormals < 5, offsetting this decrease to varying degrees, although in the majority of cases, the first five or ten flowers were less constant for five lobes than were those in the 40 to 50 flower groups.

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