STUDIES ON THE CORRELATION OF MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS: THE DEVELOP- MENT OF THE PRIMORDIAL LEAVES IN TERA- TOLOGICAL BEAN SEEDLINGS

J. ARTHUR HARRIS

Station for Experimental Evolution, Cold Spring Harbor, N. Y.

[Received February 2, 1916]

I. INTRODUCTORY REMARKS

During the past several years, I have urged that a study of the relationship between slight structural variations and the physiological characteristics of organs, or of the individuals producing them, is one of the most fundamental, although necessarily most difficult, lines of genetic research.

From the practical standpoint—considered merely in its relation to the working plans of an individual investigator—this preceding attitude has rested upon a recognition of the fact that the factor of organic evolution most in need of quantitative investigation at the present time is natural selection. Now the problem of natural selection is exceedingly comprehensive, demanding many kinds of researches for its solution. Since, however, the capacity of an individual for survival must depend upon the fitness of its several organs for performing their respective functions, individually or in coördination, it is clear that any quantitative determination of the relationship between the morphological deviations from type, to which students of genetics have hitherto so largely confined their attention, and the functional capacities of the variates has its significance in the problem of natural selection.

More fundamentally this attitude has rested upon the conviction that the morphological variations are but superficial manifestations of deeper-lying physiological—and ultimately chemical and physical—changes.

It is inconceivable that biologists will be willing to leave the problem of morphological variation without a sustained effort to locate the proximate causes in physiological, and even in chemical and physical, differentiation.

The most of the data from our work along these lines is as yet unpublished, but some phases have been treated either fully or in a pre-

liminary way. For example, it has been shown (HARRIS, 1910 a, b, 1911 a) that in *Staphylea* the capacity of the ovary for development is to some extent correlated with the number of locules and with the number and arrangement of the ovules. In unilocular fruits, reduced fertility has been shown to be associated with bilateral asymmetry in *Phaseolus vulgaris* (HARRIS 1912). There is also some, though not as yet fully convincing, evidence that seed weight is also influenced by bilateral asymmetry (HARRIS 1912 b).

In *Cercis* (HARRIS 1914 a, b) and *Phaseolus* (HARRIS 1913) capacity for seed production as measured on the mature pods, has been shown to be correlated with the absolute number of ovules laid down. The full interpretation of these results is rendered difficult because of the fact that there may be in such unilocular ovaries a differential mortality such as has already been demonstrated in *Staphylea*, but which has so far not been shown to occur (HARRIS 1909), though it is not improbable that this will sometime be done.

Finally, the death rate of transplanted seedlings has been shown (HARRIS 1912 c) to be higher in morphologically aberrant than in normal beans. These latter results have been confirmed by extensive subsequent data, as yet unpublished. At present the problem of the significance of various types of structural variation in the capacity for survival of the individual is under investigation.

The purpose of the present paper is to present the results of one attempt to determine something of the more fundamental physiological characteristics to which incapacity for survival may be due. In the seed, the primordial leaves of the bean are minute structures. If innate physiological conditions of a kind which may affect growth be associated with morphological variations, one might expect some influence of these factors to be recorded in the size or other characteristics which result from the relatively enormous expansion which these organs undergo in the course of germination and the establishment of the young seedling. This study was undertaken to determine whether the influence of such factors is discernible.

METHODS

The investigation of such a problem is simple enough in principle, but exceedingly exacting and laborious in detail. The present study has been planned for several years, but it was only recently that all of the necessary conditions could be secured at the same time.

The essentials are: (a) A large quantity of seed of a strain known

to produce variations of suitable character. (b) Greenhouse space sufficiently extensive and varied in conditions to permit the germination and subsequent culture of a number of seedlings adequately large to yield the required number of aberrant individuals in a period of time sufficiently short to give large samples of primordial leaves fairly homogeneous with respect to age. (c) Observational assistants to attend to the classification of the seedlings. While the present paper includes the results of 26 sets of determinations only, the assembling of the materials required the germination and sorting of about 100,000 seedlings.

The experimental technique adopted was exceedingly simple. Seeds of a strain of navy beans (designated by formulae involving H in published papers) which has been under observation for some years at the STATION FOR EXPERIMENTAL EVOLUTION, were germinated in sand in warm houses. Given types of variations which experience had shown to occur with the greatest frequency, were saved from the seed pans for potting in soil. These were placed in three inch pots, each with a normal individual drawn at random from the same sibship to serve as a control. After these had stood for some time in a cooler house to become established and to allow the more complete development of the laminae, samples of the leaves (exclusive of the petioles) were gathered. The age of the plants at the time of the gathering of the samples necessarily varied somewhat, both within the individual collections and from collection to collection. Practically no plants were taken before the cotyledons fell; most of them showed some growth of the terminal bud beyond the primordial leaves; some had one or in some cases two of the compound leaves well developed; none were taken in which the primordial leaves were distinctly yellow or dried because of age. All pairs of plants in which one of the individuals had apparently been injured by external causes were discarded. Plants, normal or abnormal, in which the leaves were dwarfed or crumpled without any evidence of external injury were included.

The laminae were gathered as rapidly as possible into weighed test tubes, which were again weighed and plunged into an ice and salt mixture for freezing. The gathering of the two types of plants was so carried out that there can be no source of differentiation in the collecting.

Thus, although weight and other characteristics vary from sample to sample because of age and the innumerable slight influences of significance in growth, the aberrant plants and their controls had from the very beginning as nearly as possible identical environment. However much the pairs combined in the same sample may differ among themselves,

there seems no possibility of considering that the differentiation here shown to exist between the morphologically typical and the morphologically aberrant individuals is due to any extrinsic cause.

In the absence of any knowledge of the amount of variation in the characteristics of the leaves to be investigated, it was impossible to compute in advance the size of the sample which should be taken. Accordingly 100 plants of both normals and controls was arbitrarily chosen as the size of the sample. This proved most satisfactory for technical reasons, since it supplied an amount of sap sufficient for two determinations of freezing-point and electrical conductivity. It was about as large as could be obtained without making the sample too heterogeneous with respect to age.

PRESENTATION OF DATA

Consider first the mean weight of the primordial leaves in normal and aberrant plants.

The data for all types are given in table 1. Here the mean weight per plant of leaf tissue and the difference in mean weight (abnormal mean less control mean) together with the weight index are given for each of the 26 samples. Weight index is merely the ratio of the mean weight of variants to the mean weight of their control plants.

Each of these samples includes, with exceptions to be noted below, variants of a particular type. Thus samples of class A^1 include what is probably the simplest variation in the bean seedling. Here there is merely a slight separation of the cotyledons. The amount of this separation is difficult to express quantitatively since it is to so large a degree dependent upon the absolute size of the plant. "Slightly but distinctly separated," has been the term which we have always used to designate this class. The cotyledons range in position from those which are just perceptibly not inserted on the same level to those which are perhaps two or three millimeters apart. So slight is the variation that in the classification of the seedlings, frequent discussions arose concerning the normality or abnormality of individual plants.

Notwithstanding the smallness of the morphological variation it is unquestionably correlated with physiological capacities. In each of the 9 determinations the mean weight of the plants with slightly separated cotyledons is lower than that of those in which they are on sensibly the

¹The samples are labelled serially *a* to *z*. Except as a means of identifying individual samples in comparisons between different tables the reader need concern himself with only the classes, which are designated by capital letters.

TABLE I

Sample	Mean Weight per Plant for Abnormal	Mean Weight per Plant for Normal	Difference	Weight Index
<i>Aa</i>	.415	.520	— .105	.798
<i>Ab</i>	.469	.552	— .083	.850
<i>Ac</i>	.415	.497	— .082	.835
<i>Ah</i>	.401	.473	— .072	.848
<i>Ak</i>	.447	.575	— .128	.777
<i>An</i>	.363	.456	— .093	.796
<i>As</i>	.382	.483	— .101	.791
<i>At</i>	.333	.435	— .102	.766
<i>Ax</i>	.311	.392	— .081	.793
<i>Bw</i>	.468	.595	— .127	.787
<i>Ca</i>	.377	.483	— .106	.781
<i>Cl</i>	.423	.547	— .124	.773
<i>De</i>	.579	.631	— .052	.918
<i>Df</i>	.597	.640	— .043	.933
<i>Dg</i>	.455	.541	— .086	.841
<i>Di</i>	.539	.582	— .043	.926
<i>Dm</i>	.470	.529	— .059	.888
<i>Do</i>	.434	.510	— .076	.851
<i>Dp</i>	.458	.495	— .037	.925
<i>Dq</i>	.465	.517	— .052	.899
<i>Du</i>	.506	.530	— .024	.955
<i>Dv</i>	.401	.446	— .045	.899
<i>Ej</i>	.391	.503	— .112	.777
<i>Er</i>	.382	.536	— .154	.713
<i>Xy</i>	.364	.424	— .060	.858
<i>Xz</i>	.304	.428	— .124	.710

same level. The weight index ranges from .766 to .850 with an average value of .806.

Cotyledon position in dicotyledonous bean seedlings is a character showing wide quantitative variations. Insertions may range from the same level to very widely separated, say two or three centimeters, depending upon the degree of elongation of the axis. Plants with the higher degrees of separation are correspondingly rare. In only one case, *Bw*, was I able to secure 100 plants of suitable age in which the cotyledons were more widely separated than in the class *A* plants just described. Again the abnormal plants show a lower average laminar development than the normal controls. The weight index is .787. Only two of the 9 indices of the class *A* plants just described are as low as this, and their mean value is .806. It is not, however, possible to assert on the basis of this single determination that the weight index is signifi-

cantly lower than in the plants with the same kind but smaller degree of abnormality, discussed above (class *A*).

A class of structural variation which perhaps represents a greater deviation from the normal condition than the preceding is that in which the number of cotyledons is three. These may be arranged in a whorl or somewhat separated in insertion. It has not been possible to classify more minutely with respect of position when the cotyledons are three instead of two in number. In such seedlings the primordial leaves may be either normal or abnormal. In two samples, class *C*, it was possible to secure plants which were abnormal in cotyledon number (tricotyledonous) but quite normal in number and insertion of the primordial leaves. The results may be compared with those based upon plants which deviate from normality by the separation of their two cotyledons only and with those based upon plants with three cotyledons and three primordial leaves in a regular whorl, class *D*.

Both of these weighings show a slighter development of primordial tissue in the abnormal plants. The indices are both lower than the average of class *A* plants, and but 2 of the 9 samples of that class show relative weights as low as these two. Apparently, therefore, the plants with three cotyledons and two primordial leaves are less capable of developing these leaves than are those with the normal number but with slight separation of the cotyledons. Larger series of determinations would, however, be necessary fully to establish this point.

Turn now to a consideration of the plantlets which have three cotyledons, as in the class just discussed, but a whorl of three normally-formed primordial leaves instead of two. There are ten such determinations. *In every individual sample the weight of the three leaves of the trimerous plant is on the average lower than that of the two of the normal dimerous plant.* The weight index varies from .841 to .955 with an average value of .904. Had the results of the weighing of these 5000 leaves shown that when in variation three primordial leaves occur instead of the normal two the mean weight of an individual leaf is lower than that of an individual leaf of a normal plant, there would have been no valid cause for surprise. What the determinations actually show is that, notwithstanding the fact that the number of leaves has *increased* fifty percent, their total weight has *decreased* ten percent.

The increase in leaf number is, however, not without significance. Without exception the ten indices are higher than either of the two based upon plants which have three cotyledons but a normal pair of leaves. Of the ten determinations recorded in this class there is only a single

one which falls below the maximum value of the 9 based on plants of class *A*. The average index is about ten percent higher, i.e., .904 as compared with .806.

If the weight indices be calculated on the basis of the mean weight of individual leaves instead of upon the basis of the mean weight of leaf tissue per plant, the results are quite different. Thus:—

<i>Class A plants</i>		<i>Class D plants</i>	
Sample	Index	Sample	Index
<i>Aa</i>	.798	<i>De</i>	.612
<i>Ab</i>	.850	<i>Df</i>	.622
<i>Ac</i>	.835	<i>Dg</i>	.560
<i>Ah</i>	.848	<i>Di</i>	.617
<i>Ak</i>	.777	<i>Dm</i>	.592
<i>An</i>	.796	<i>Do</i>	.567
<i>As</i>	.791	<i>Dp</i>	.617
<i>At</i>	.766	<i>Dq</i>	.600
<i>Ax</i>	.793	<i>Du</i>	.636
		<i>Dv</i>	.600
<hr/> Means		<hr/>	
	.806		.602

Finally, it was possible to secure two fairly satisfactory samples of plants with axes slightly broadened or much fasciated, class *E*. Such individuals were either normal or abnormal in number and arrangement of cotyledons and primordial leaves. Generally the cotyledons were more than two. This was almost invariably the case with the primordial leaves which varied in number, position, size, fusion and so on. In many instances these fasciated plants appear to the eye more vigorous than the normal individuals of the same pedigree. I would not therefore have been surprised to find them producing greater weight of leaf substance than the normal plants. The weight indices show that notwithstanding the larger number of leaves which they produce, they develop about 25 percent less leaf tissue than the normal controls.

The remaining collections, class *X*, are mixtures of abnormal plants made in cases in which materials were insufficient for determinations to be based upon a single type only. They furnish their contribution to the problem of the relationship between morphological deviation and physiological capacity, but cannot be used in a comparison between different morphological types.

Taking the materials as a whole it is clear that in the 26 samples (each of 100 abnormals and their controls) the mean weight of leaf tissue developed in the case of the morphologically abnormal plants is in every instance lower than that of the normal controls. The weight index, which represents merely another and in some respects a better

method of expressing the same relationship, is in every case less than unity. The mean value for the 26 series is .834.

Turn now to a consideration of a series of approximate determinations² of the freezing-point lowering and electrical conductivity of the sap of these leaves.

After weighing, the tubes of leaf tissue were plunged into an ice and salt mixture as a preliminary to the extraction of sap (GORTNER and HARRIS 1914). Freezing-point lowering was determined by the well-known BECKMANN method. The depressions, deltas, have been corrected for supercooling.

The depression of the freezing-point, Δ , of the several samples are given in table 2. These serve as a measure of the concentration in

TABLE 2

Sample	Delta for Abnormal Plants	Delta for Normal Plants	Difference	Delta Index
<i>Aa</i>	.577	.597	— .020	.966
<i>Ab</i>	.614	.636	— .022	.965
<i>Ac</i>	.664	.653	+ .011	1.017
<i>Ah</i>	.537	.524	+ .013	1.025
<i>Ak</i>	.616	.639	— .023	.964
<i>An</i>	.597	.658	— .061	.907
<i>As</i>	.653	.682	— .029	.957
<i>At</i>	.651	.672	— .021	.969
<i>Ax</i>	.601	.626	— .025	.960
<i>Bw</i>	.593	.593	+ .000	1.000
<i>Cd</i>	.646	.649	— .003	.995
<i>Cl</i>	.607	.620	— .013	.979
<i>De</i>	.618	.623	— .005	.992
<i>Df</i>	.611	.593	+ .018	1.030
<i>Dg</i>	.625	.610	+ .015	1.025
<i>Di</i>	.605	.598	+ .007	1.012
<i>Dm</i>	.618	.616	+ .002	1.003
<i>Do</i>	.681	.653	+ .028	1.043
<i>Dp</i>	.569	.600	— .031	.948
<i>Dq</i>	.559	.565	— .006	.989
<i>Du</i>	.634	.604	+ .030	1.050
<i>Dv</i>	.702	.681	+ .021	1.031
<i>Ej</i>	.552	.587	— .035	.940
<i>Er</i>	.555	.586	— .031	.947
<i>Xy</i>	.561	.539	+ .022	1.041
<i>Xz</i>	.565	.592	— .027	.954

²I am unable to give the experimental error of the determinations but it is relatively large. The instruments and methods necessarily used were not the most refined. Much of the work was carried out during a period when I was unable to observe all the precautions I would have liked. For the most part the constants given are averages of two independent determinations.

molecules and ions of both electrolytes and non-electrolytes in the sap of the leaf tissue. The third decimal place is certainly not significant but is given for convenience in determining the difference between the samples and their controls.

The depression for both teratological and normal plants shows considerable variation from sample to sample, just as mean leaf weight does. This is to be expected because of differences in age and because of the numerous uncontrollable growth factors. The differences between the constants for the morphologically abnormal and the morphologically normal plants are slight. They are sometimes positive and sometimes negative in sign.

Only classes *A* and *D* need be considered in detail. For class *A* there are 2 samples in which the abnormal plants show a higher concentration of solutes as compared with 7 in which they show a lower concentration than their controls. The average of the 2 positive differences is $+.012$, of the 7 negative differences $-.029$, and of all 9 differences $-.020$. Or, in terms of indices, the two cases in which concentration is higher in the abnormal plants give an average of 1.021 , as compared with 0.955 in the remaining 7 and 0.970 for the 9 determinations.

For class *D* plants the condition is just reversed. There are 7 determinations in which the abnormal plants show a higher concentration to 3 in which they show a lower concentration than the controls. The averaged results are:

	Number of Cases	Mean Difference	Mean Index
Concentration higher	7	$+.017$	$-.028$
Concentration lower	3	$-.014$	0.976
Total samples	10	$+.008$	1.012

For the materials as a whole the average differences are $+0.015$ for the 11 positive values, -0.023 for the 15 negative values and -0.007 for all 26 differences.

For electrical conductivity expressed in reciprocal ohms, as a measure of the concentration of dissociated electrolytes, very similar conditions prevail. The values differ considerably from collection to collection. Between the abnormal and the normal plants of a sample the differences in conductivity are sometimes positive and sometimes negative in sign.⁸

⁸ Conductivities are recorded to five places to facilitate obtaining the signs of the differences in all cases, not because the final or even the fourth place is regarded as certainly trustworthy in every instance.

TABLE 3

Sample	Conductivity for Abnormal Plants	Conductivity for Normal Plants	Difference	Conductivity Index
<i>Aa</i>	.01415	.01444	— .00029	.980
<i>Ab</i>	.01455	.01506	— .00050	.967
<i>Ac</i>	.01560	.01547	+ .00013	1.009
<i>Ah</i>	.01314	.01308	+ .00005	1.004
<i>Ak</i>	.01390	.01415	— .00025	.982
<i>An</i>	.01415	.01464	— .00049	.966
<i>As</i>	.01343	.01373	— .00030	.978
<i>At</i>	.01280	.01338	— .00058	.957
<i>Ax</i>	.01222	.01213	+ .00010	1.008
<i>Bw</i>	.01293	.01291	+ .00003	1.002
<i>Cd</i>	.01513	.01549	— .00036	.977
<i>Cl</i>	.01332	.01348	— .00016	.988
<i>De</i>	.01488	.01503	— .00015	.990
<i>Df</i>	.01450	.01464	— .00015	.990
<i>Dg</i>	.01455	.01473	— .00018	.988
<i>Di</i>	.01418	.01429	— .00011	.992
<i>Dm</i>	.01401	.01409	— .00008	.994
<i>Do</i>	.01497	.01479	+ .00018	1.012
<i>Dp</i>	.01395	.01395	— .00000	1.000
<i>Dq</i>	.01301	.01322	— .00021	.984
<i>Du</i>	.01359	.01309	+ .00051	1.039
<i>Dv</i>	.01392	.01354	+ .00039	1.028
<i>Ej</i>	.01330	.01330	+ .00000	1.000
<i>Er</i>	.01303	.01295	+ .00008	1.006
<i>Xy</i>	.01237	.01201	+ .00037	1.030
<i>Xz</i>	.01260	.01213	+ .00047	1.039

For the larger groups the values, summarized from table 3, are:

	Number of Cases	Mean Difference	Mean Index
<i>For class A plants</i>			
Concentration higher	3	+ .00009	1.007
Concentration lower	6	— .00040	0.972
All plants	9	— .00024	0.983
<i>For class D plants</i>			
Concentration higher	3	+ .00036	1.026
Concentration lower	7	— .00013	0.991
All plants	10	+ .00002	1.002
<i>For all abnormals</i>			
Concentration higher	11	+ .00021	1.016
Concentration lower	15	— .00025	0.892
All plants	26	— .00006	0.997

These differences, like those for the depression of the freezing-point, Δ , are so slight that no final significance is to be attached to them. All that tables 2 and 3 demonstrate is that if there are differences in the concentration of the solutes of the cell sap of the leaves of teratological and normal plants they are of a very slight order indeed. Nevertheless it must be noted that for both constants of sap properties somewhat more than half of the determinations show a lower concentration of solutes in the juices of the tissues of the abnormal leaves, and that for the materials as a whole the average concentration is lower in the abnormal plants.

CONCLUDING REMARKS

In the preceding section it has been shown that the weight of primordial leaf tissue developed by morphologically aberrant seedlings of *Phaseolus vulgaris* is on the average less than that produced by normal controls grown under conditions as nearly as possible comparable.

So far as determined, a reduction in the volume of primordial leaf tissue occurs irrespective of the kind of abnormality. The type of abnormality does, however, determine within wide limits the degree of reduction in the amount of leaf tissue.

A first attempt has been made to ascertain by means of the conductivity and freezing-point methods, whether there are differences in the concentration in molecules and ions in the cell sap of the leaves of teratological and normal plants. The evidences suggest that there may be a lower concentration of both electrolytes and total solutes in the tissue fluids of the teratological plants. If, on more extensive investigation this should prove to be true, it would be quite in agreement with the results for leaf weight, indicating that the morphologically aberrant individuals have a smaller capacity for absorbing electrolytes or for synthesizing electrolytes and non-electrolytes. The differences are, however, so slight and so variable that further and more refined determinations will be necessary to demonstrate the existence of any relationship. So far as data are on the table, they demonstrate merely that no clear difference exists in the properties of the sap of the leaf tissue of the two types of leaves.

LITERATURE CITED

- GORTNER, R. A., and HARRIS, J. A., 1914 Notes on the technique of the determination of the depression of the freezing-point. *Plant World* **17**:49-53.
- HARRIS, J. ARTHUR, 1909 Is there a selective elimination of ovaries in the fruiting of the Leguminosae? *Amer. Nat.* **43**:556-559
- 1910 a On the selective elimination occurring during the development of the fruit of *Staphylea*. *Biometrika* **7**:452-504.
- 1910 b The selective elimination of organs. *Science N.S.* **32**:519-528.
- 1911 a Further observations on the selective elimination of organs in *Staphylea*. *Zeitschr. f. ind. Abst. u. Vererb.* **5**:173-188.
- 1911 b The measurement of natural selection. *Pop. Sci. Mo.* **78**:521-538.
- 1912 a On the relationship between bilateral asymmetry and fertility and fecundity. *Arch. f. Entwicklungsmech. Orman.* **85**:500-522.
- 1912 b On the relationship between the bilateral asymmetry of the unilocular fruit and the weight of the seed which it produces. *Science N. S.* **86**:414-415.
- 1912 c A simple demonstration of the action of natural selection. *Science N. S.* **36**:713-715.
- 1913 On the relationship between the number of ovules formed and the capacity of the ovary for developing its ovules into seeds. *Bull. Torr. Bot. Club* **40**:447-455.
- 1914 a On the relationship between the number of ovules formed and the number of seeds developing in *Cercis*. *Bull. Torr. Bot. Club* **41**:243-256.
- 1914 b Further observations on the relationship between number of ovules formed and number of seeds developing in *Cercis*. *Bull. Torr. Bot. Club* **41**:533-549.