THE GENETIC COEFFICIENTS OF SPECIFIC DIFFERENCE

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For the precise study of evolution of populations, races, or species, nearly every problem sooner or later requires some measurement of the morphological divergencies in the groups under observation. This is equally true and the problem is fundamentally the same whether one be studying very closely related species of *Drosophila* (Dobzhansky and Mather, '39), varieties of gall wasps (Kinsey, unpublished), fields of irises (Anderson, '36a), or the races of man (Pearson, '26, and various other authors). It is usually taken for granted in such studies that any measurable feature or features of the organism will serve equally well as a measure of likeness if only the records be made with care and treated with the precise methods of biometry. Improvements have recently been made by considering differences in groups of measurements, the data being combined crudely (Anderson, '36a, '36b, Anderson and Hubricht, '38) or by refined biometrical techniques (Fisher, '36b).

These methods are all based on the tacit assumption that species differences are expressed more or less at random. A study of such differences has convinced us that their morphological nature renders these methods relatively inefficient. Species do not differ in a random manner. They differ in a peculiar and subtle way. If any two closely related species of the flowering plants are examined critically it will be found that they differ as a whole by two sets of harmonically integrated tendencies (Anderson and Whitaker, '34). Such a conclusion, however, is of little use in quantitative work. In section I, therefore, there is developed a precise mathematical

expression for the difference between "two sets of harmonically integrated tendencies." The application of this formula is illustrated in section II, where an attempt is made to analyze the differences between *Nicotiana alata* and *N. Langsdorffii* and to show how, from an estimate of their "genetic co-efficients," an efficient measure of their total difference could be developed.

I. A GENERAL FORMULA FOR THE EFFICIENT MEASUREMENT OF SPECIFIC DIFFERENCES

It might seem impossible to formulate any mathematical definition of species differences broad enough to apply to organisms as different as flowering plants, insects, and vertebrates. A little reflection, however, will remind one that the gene-chromosome-cell relation is fundamentally the same in these various organisms and that species differences, in so far as they rest on the gene-chromosome-cell system, may be expected to exhibit certain general features.

Closely related species or races may be conceived as made up of a large number of characters, the number considered in any particular instance depending upon the viewpoint of the observer. Any two closely related species, however, will have the same sets of characters which differ only in their proportionate development. In studying races of mankind, for instance, there might be considered the head, the neck, the trunk, the arms, and the legs of the two races. If the set of characters were subdivided into such categories as fingers, ears, etc., it would still be possible to observe the same set in both races.

We may therefore define the gross morphology of any organism as being the sum of a set of characters: Organism = A + B + C + D + E + F + ... + N. In so far as species differences rest in the germ-plasm, the basic differences between the two species will not be differences in these characters but in the germ-plasm which give rise to them, and they can be thought of as made up of a set of differences between corresponding factors of the germ-plasm. These factors in the germ-plasm we shall write a, b, c, d, e, ... n for one species, and a′, b′, c′, d′, e′, ... n′ for the other. Some of these may relate to proc-

esses so general that they are expressed in every character (as, for instance, a gene affecting cell division or wall formation). For such factors we shall use the first letters of the alphabet and we may write the first species as: (abc .....)A + (abc .....)B + (abc .....)C + (abc .....)D + ... + (abc .....)N, while the second species will be written: (a′b′c′ .....)A + (a′b′c′ .....)B + (a′b′c′ .....)C + (a′b′c′ .....)D + ... + (a′b′c′ .....)N. The dots within the parentheses represent additional factors affecting all the characters. Other factors will affect only similar characters, as, for instance, the leaf and the calyx in flowering plants, or hand and foot in vertebrates. For them we may use the middle letters of the alphabet. There are probably also elements in the germplasm which affect only single characters. If we use letters at the end of the alphabet for them, then the total morphological difference between two related species is described by the following mathematical expression:

(abc ... m .. x ..)A + (abc ... m .. y ..)B + (abc ... n .. z ..)C + ... + (abc ... p .. w ..)N − (a′b′c′ ... m′ .. x′ ..)A + (a′b′c′ ... m′ .. y′ ..)B + (a′b′c′ ... n′ .. z′)C + ... + (a′b′c′ ... p′ .. w′ ..)N.

From this it follows that a set of observations upon A or upon A and B will probably be an inefficient way of getting at fundamental differences between the two species. That is to say, instead of comparing two races of men by their skulls alone, or two species of *Acer* by their leaves, we should first attempt to determine the most efficient way of measuring the coefficients which affect skull, trunk, and appendages in man, or leaf, stem, and inflorescence in *Acer*. What is needed is the most efficient way of measuring (a − a′), (b − b′), (c − c′), ... (n − n′). These genetic coefficients of specific difference (a vs. a′, b vs. b′, c vs. c′, etc.) cannot be determined from casual inspection. While their determination is a much more simple matter in the flowering plants than in the insects or vertebrates, it will even there require detailed observation and experiment. How to measure any particular specific difference is a research problem which should be undertaken before one proceeds to the actual measurement.

II. AN ESTIMATE OF THE GENETIC COEFFICIENTS WHICH DIFFERENTIATE *NICOTIANA ALATA* FROM *N. LANGSDORFFII*

The species chosen for comparison were *Nicotiana alata* and *N. Langsdorffii.* They were selected because (1) they are easily grown for observation and experiment, (2) a large body of genetic and cytological data is already at hand concerning their behavior in crosses and back-crosses (East, '16, Sachs-Skalinska, '21, Brieger, '35, Smith, '37, Avery, '38, Anderson, '39), (3) an estimate of their genetic coefficients was desired as the basis for analysis in further crosses. *Nicotiana alata* is the night-blooming species with large white flowers, known to gardeners as *N. affinis. N. Langsdorffii* is a smaller, chunkier species, with bright green flowers and blue pollen. Representative flowers of each are illustrated in plate 24, A–C. Seed of *N. alata* was obtained from the Palmer Seed Company of St. Louis. Some of the plants bore pale pink corollas, probably the result of hybridization in cultivation with X *Nicotiana Sanderae (= N. alata X N. Forgetiana).* The strain of *N. Langsdorffii* was kindly supplied by Dr. H. H. Smith of the U. S. Department of Agriculture. The known facts of the relationship and distribution of the two species have been summarized by Avery ('38). The points which concern us here are that both species are diploid members of the 9-chromosome group of *Nicotiana,* and that they are both native (or are at least widely distributed) in a large region in central South America. From a study of the meiotic configurations of their hybrids Avery concluded that the gross differences in their chromosome complements were confined to two translocations in three pairs of chromosomes. Like some of the evidence submitted below, this fact supports (though it does not prove) Anastasia's speculation ('14) that *N. Langsdorffii* may be the result of a cross between *N. alata* or a closely related species and some such member of the 24-chromosome group as *N. rustica,* by which a few segments of *rustica* germ-plasm became incorporated in an *alata* genom (Avery, '38). If this is indeed the relationship between *N. alata* and *N. Langsdorffii,* the case, while exceptional, is not unique in our opinion. There

are a number of genera of flowering plants in which the morphological resemblances between the species would indicate similar relationships.

1. Cell size.—In searching for the fundamental genetic coefficients which differentiate these two species, one of the most obvious places to look is the cell itself. If there are outstanding differences in cell size, cell uniformity, or in the development of the cell wall, they should be comparatively easy to detect. An inherent cell-size difference, for instance, should manifest itself in a consistently larger size of one species, even in those organs in which there are no obvious differences in proportion. Even a superficial examination will show that

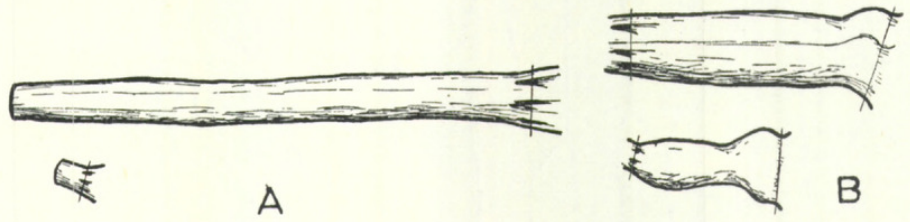

Fig. 1. A, corolla-tube of *Nicotiana alata* (above) and of *N. Langsdorffii* (below); B, corolla-throat of *N. alata* (above) and of *N. Langsdorffii* (below). All figures drawn to the same scale.

Nicotiana alata is generally larger throughout than is *N. Langsdorffii.* The shape differences in the corolla are confined to the base of the tube and the limb. The throat of the corolla, although complex in shape, is of practically the same proportion in the two species, and is roughly half again to twice as large in *N. alata* as in *N. Langsdorffii* (pl. 24, and fig. 1, B). The pedicels, the cross-section of the style, the capsule, and the seeds show the same relationship. Histological examination shows that the surmise of a fundamental difference in cell size is probably correct. While measurements of whole tissues were not undertaken, examinations were made in all those organs which seemed to have about the same proportions. Camera-lucida drawings are presented in fig. 2. It will be noted that, in each, the cells of *N. alata* are larger than those of *N. Langsdorffii* and that in each the ratio of their diameters

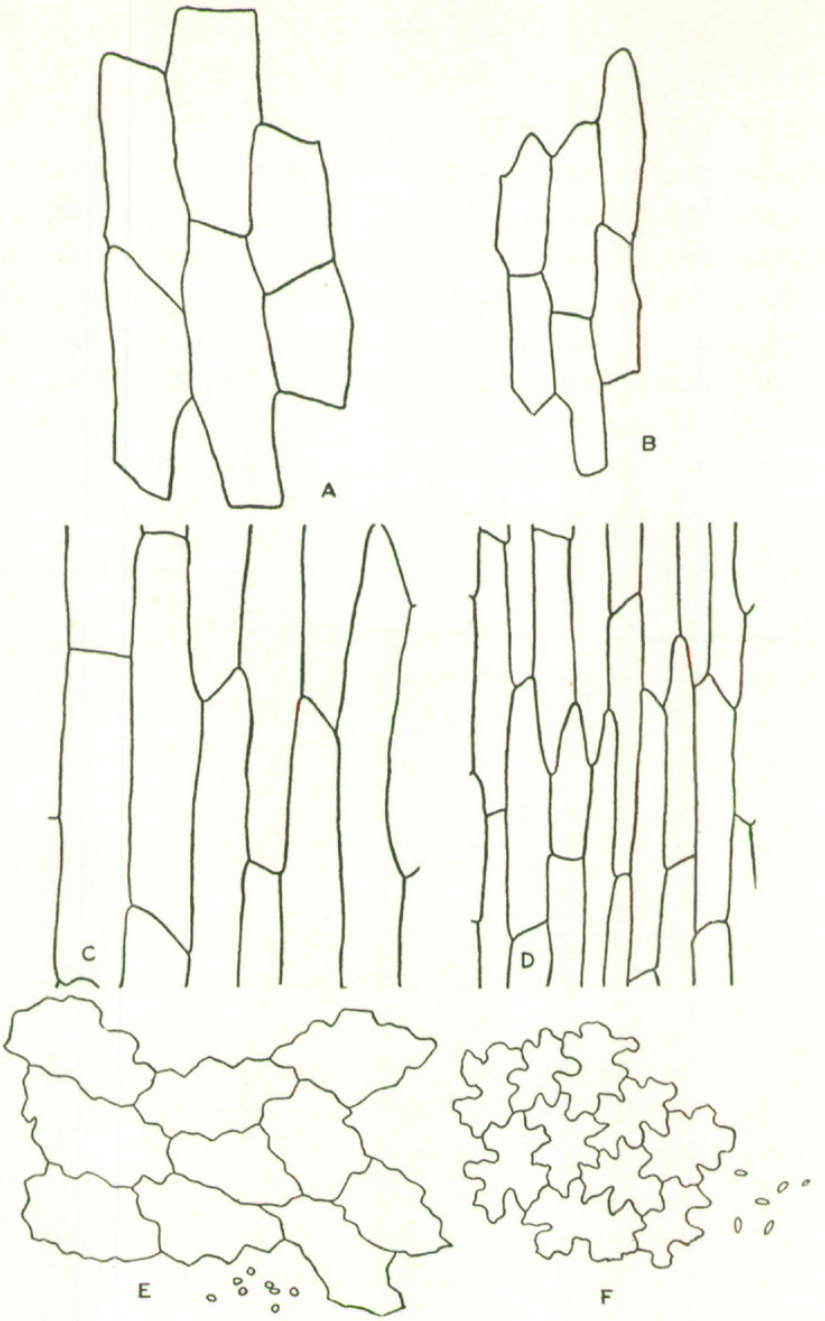

Fig. 2. Histological details to show relative size of cells in *Nicotiana alata* and *N. Langsdorffii:* epidermal cells from base of corolla-tube, (A) in *N. alata,* (B) in *N. Langsdorffii;* epidermal cells from corolla-throat, (C) in *N. alata,* (D) in *N. Langsdorffii;* ten epidermal cells from corolla-limb, (E) in *N. alata,* (F) in *N. Langsdorffii.* The plastids drawn in E and F show relative size, but not relative number or distribution.

is roughly from 1:1.5 to 1:2. Furthermore, this ratio agrees with the size differences of the organs concerned. Note particularly the pedicels, the corolla-throats, the pollen, and the seeds (fig. 3 and pl. 24).

As a working hypothesis we may therefore conclude that one of the fundamental differences between *N. alata* and *N. Langsdorffii* is cell size, and that it is apparently expressed throughout the organism. Its expression is certainly modified by localized differences in cell elongation, as will be shown below, and perhaps by differences in cell number, though we have as yet little definite information on that point.

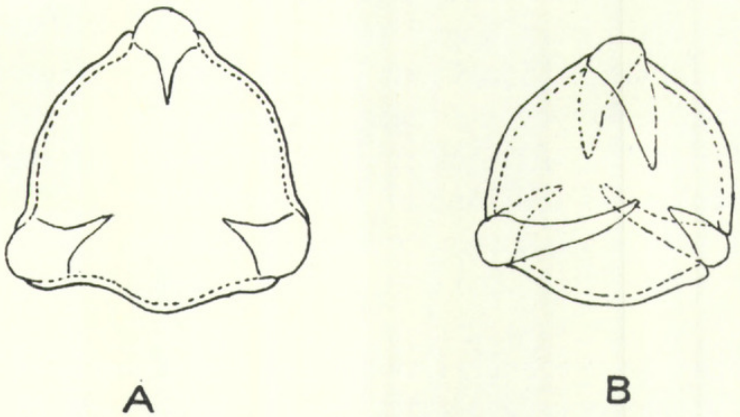

Fig. 3. Pollen grains of (A) *Nicotiana alata,* and (B) *N. Langsdorffii.*

2. Cell elongation.—The most striking difference in flower shape between the two species is the constricted portion of the corolla-tube below the point where the stamens are inserted. In *Nicotiana Langsdorffii* this is so short that it cannot be seen without removing the calyx. In *N. alata* it is much longer than the throat (pl. 24, A, C, and fig. 1, A). Histological examination showed that the difference is mainly due to cell elongation. Allowing for the basic difference in cell size (see above) the cells of the tube in *N. alata* are proportionately no wider than those in *N. Langsdorffii* though they are many times as long (Nagel, '39). It seemed probable that such a difference should be expressed elsewhere throughout the plant, and even a cursory examination showed this to be the case. *Nicotiana alata* is not only a somewhat larger plant

than *N. Langsdorffii;* it has a general tendency to be somewhat more elongated. It has narrower leaves (largely due to more elongated petioles), longer internodes, narrower bracts, longer calyx-lobes, a much longer style, and a more pointed ovary, resulting in elongate lobes of the ripened capsule (pl. 24, D, E). It seemed probable that all of these correlated differences rest on a difference in the mechanism of cell elongation. This point has very kindly been investigated by Miss Nagel, whose results are reported in the accompanying paper. She finds that there is a basic difference in the auxin response of the two species. *Nicotiana Langsdorffii* apparently inactivates auxin very readily and therefore shows little or no response even when it is supplied artificially in various ways. *Nicotiana alata,* on the other hand, does not inactivate it so readily and, in stem, leaf, and flower, shows even greater elongation when additional auxin is supplied artificially. It therefore seems quite definitely established that one of the differentiating genetic coefficients affects the auxin mechanism, probably by bringing about greater auxin inactivation in one species than in the other.

It seems quite probable that several of the coefficients listed below may be only accessory manifestations of this same auxin difference. This is particularly true of number 3, geotropic response, and number 4, leaf-vein angles.

3. Geotropic orientation of appendages.—Appendages of the axis, and its own branches, diverge at a more acute angle in *Nicotiana alata* than in *N. Langsdorffii.* This angle divergence is roughly the same in leaves, pedicels, bracts, and branches of the inflorescence (fig. 4). It has been well established that the geotropic response of flowering plants is accomplished through auxin regulation (Dolk, '36). Whether or not the difference in appendage orientation is due to the same auxin-mechanism difference as that affecting corolla-tube elongation we have as yet no means of proving.

4. Leaf-vein angles.—The angles made by the side-veins with the midrib of the leaf are also more acute in *N. alata* than

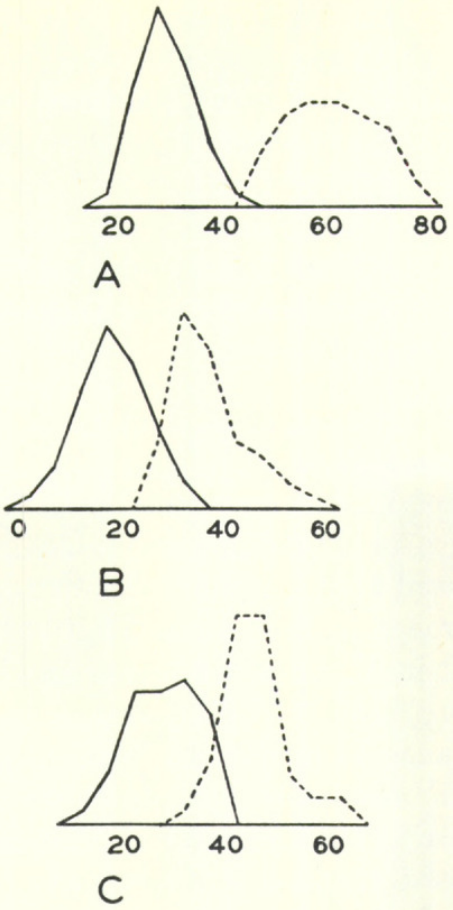

Fig. 4. Frequency distributions showing angle of divergence of (A) leaf, (B) flowering pedicel, and (C) branch of the inflorescence. The solid line, in each case, represents *Nicotiana alata*, the broken line, *N. Langsdorffii*. The numbers along the base lines represent the angles of divergence, in degrees.

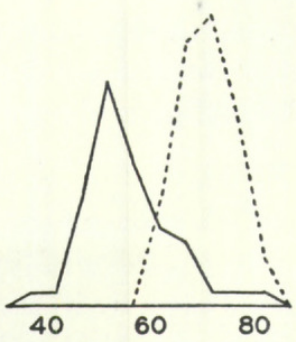

Fig. 5. Frequency distributions showing angle of divergence of the secondary vein near the base of the leaf blade, in *Nicotiana alata* (solid line) and *N. Langsdorffii* (broken line). The numbers represent the angles, in degrees.

in *N. Langsdorffii* (fig. 5). While it is probable that this difference is related to auxin concentrations, further experimentation will be required to discover its relation to geotropism and elongation in the appendages.

5. Plastid color.—The most conspicuous difference between the two species is the color of the flowers. The corollas of *N. alata* are a clear ivory-white within, somewhat tinged with green on the outside. Those of *N. Langsdorffii* are bright green

on both sides. Microscopical examination shows this difference to reside in the plastids, which are ivory in the former and green in the latter. While this difference is most extreme in the flower it is also expressed in other parts of the plant, notably in the midribs of the leaves and in the pedicels. These are ivory at maturity in *N. alata* and green in *N. Langsdorffii*. We therefore conclude that one of the genetic coefficients which differentiate the two species is the ability to develop ivory rather than green plastids under certain conditions.

6. Peripheral foliar development.—One of the most striking differences between the flowers of *N. alata* and *N. Langsdorffii* occurs in the corolla-limb. In the former species it is larger and deeply lobed; in the latter, small and almost unlobed. The difference in cell size, discussed above, would account for not more than half of the difference in limb size. That there is evidently a genetic coefficient in *N. alata* producing continued development of the marginal tissue in foliar organs is suggested by a comparison of the leaves of the two species. Those of *N. Langsdorffii* are characteristically flat. In those of *N. alata* the margin has developed to such an extent that it cannot be accommodated in a flat position and is strongly waved. We therefore suggest that one of the differentiating genetic coefficients we are seeking affects the development of the margin in leaf and corolla.

7. Basal foliar development. A further conspicuous difference between the species is in the shape of the corolla limb, which is deeply lobed in *N. alata* and so slightly lobed in *N. Langsdorffii* that the limb sometimes has a slightly greater diameter at the sinuses than at the apex (which can still be recognized, however, by the veining pattern). Part of this difference in shape is a physiological necessity of the greater size and is not due to specific shape differences. It has already been shown (Anderson, '39) that in the genicly uniform F_1 between the two species there is a correlation of .3105 ± .1077 between the degree of lobing and the limb width. An examination of the limb offers a simple explanation of this correlation.

The main vein is down the center of the lobe, and it might be expected that with increased growth of the limb there must of necessity be a greater increase proportionately at those points near the food supply (the tips of the lobes) than at those points which are remote from the food supply (the sinuses). There is evidence, however, that there are factors in *Nicotiana alata* making for accentuated lobes other than those concomitant with the increase in size. The F_2 correlations between lobing and limb width are much greater ($.7186 \pm .0300$) than those of the F_1, indicating a genetic correlation as well as a purely physiological one. Furthermore, second-generation hybrids with limbs of the same size differ among themselves in the amount of lobing of the corolla. *Nicotiana alata* therefore differs from *N. Langsdorffii* not only in the size of its limb but in a tendency for the limb to grow more towards the tip and less towards the base.

It seems not impossible that this same tendency may also operate in the other foliar organs. The leaves of the two species differ in length of the petiolar portion (as has been discussed above) and in shape of the basal portion of the blade, which is proportionately wider in *N. Langsdorffii.* If two leaf blades of about the same size and age are selected and laid side by side it will be seen that their tips are very similar and that most of the difference in blade shape is due to the wider base. The leaf of *N. Langsdorffii* is furthermore more decurrent on the stem than is that of *N. alata.* As a basis for further experiment we would therefore suggest that one of the genetic coefficients distinguishing the two species is a factor for greater basal development in foliar organs. Its chief effect in *N. Langsdorffii* is to make the blade proportionately broader at the base and, by exerting a similar effect upon corolla-lobes, to lessen the lobing of the corolla. The evidence for such a coefficient is much more speculative than that for the coefficients previously discussed.

8. Pollen color.—The pollen of *N. Langsdorffii* is bright blue, that of *N. alata* is ivory-colored. Smith has shown ('37) that the production of blue pollen is due to two complementary

genes which are independent of the gene for green plastid color.

9. Time of blooming.—The flowers of *N. alata* begin to open late in the afternoon and close, as if wilted, during most of the day. While we have made no precise experiments, this is apparently correlated with both light and temperature. On a dark day, or indoors, the flowers of *N. alata* may remain more or less expanded throughout the day. *Nicotiana Langsdorffii,* on the other hand, is a day-blooming species, though it wilts in strong sunshine even more readily than other day-blooming Nicotianas. It seems possible that this difference between the species may be another expression of the plastid difference discussed above. If this be true, it should be possible to establish the fact by a careful study of second-generation and back-cross individuals.

10. Scent.—The flowers of *N. alata* are delightfully scented, particularly when they first expand in the early evening. Those of *N. Langsdorffii* have little or no odor.

11. Inflorescence.—Typical inflorescences of each species are diagrammed in fig. 6. They exhibit at least two kinds of difference between the two species: degree of branching, and determinate vs. indeterminate nodes. *Nicotiana Langsdorffii* shows a much higher degree of branching than does *N. alata.* It is difficult to score definitely because in both species the amount of branching is affected by the food supply. Starved in a two-inch pot even *N. Langsdorffii* will have a simple stem. When grown in four- or five-inch pots, however, it always shows numerous well-developed secondary axes and at least a few of the third and fourth order. *Nicotiana alata* often shows only a few secondary and no tertiary axes.

Nicotiana alata is apparently indeterminate, but there is no transparent relation between flowers and bracts. In *N. Langsdorffii* every axis, whether primary, secondary, or of a higher order, is terminated by a flower. The terminal flower on the primary axis is the first to bloom, followed by those terminating the two upper secondary axes. These facts would indicate that the inflorescence is in part truly determinate. On

the other hand, these terminal flowers are not subtended by bracts, but small bracts, usually without flowers, occur a short way up each of the secondary axes. This might indicate that the terminal flowers are falsely determinate. Whether the de-

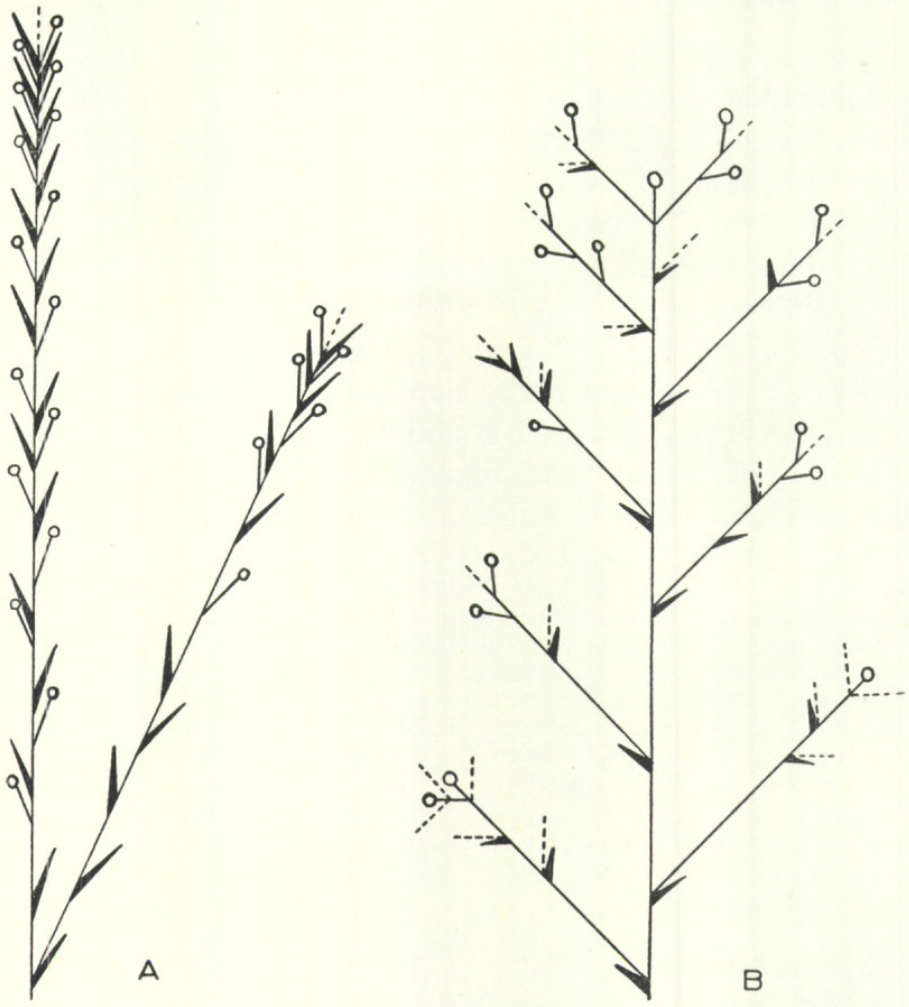

Fig. 6. Inflorescence diagrams of (A) *Nicotiana alata*, and (B) *N. Langsdorffii*. The angles of divergence of leaves, pedicels, and branches are average ones for the two species. No attempt is made to show relative length of internodes, leaves, or pedicels. Broken lines indicate continuation of the axes.

terminateness of *N. Langsdorffii* is affected by coefficients which are expressed elsewhere in the organism cannot be ascertained without further experiment. From what is known about such matters it would seem highly possible that the degree of branching might be affected by the auxin mechanism.

Cell shape.—There are various differences in cell shape between the two species, particularly in the cells of the epidermis. Not enough work has been done to show whether or not these differences can be reduced to differences in one or a few basic coefficients.

Zygomorphy.—The flowers of both species are slightly zygomorphic, though in *N. alata* it is the corolla-limb which shows its bilateral symmetry most strikingly, while in *N. Langsdorffii* the expression of this tendency is stronger in the corolla-tube and throat. It is quite probable that these may be further manifestations of the basis for the vein-angle and leaf-angle differences.

In addition to the differences discussed above there are a number of minor ones whose expression is apparently limited to a single organ. Further genetical and physiological experimentation may show that some of these are further effects of the coefficients described above.

TABLE I

SUMMARY OF THE GENETIC COEFFICIENTS DIFFERENTIATING *N. ALATA* FROM *N. LANGSDORFFII*. "x," ORGANS IN WHICH THE ACTION OF THE GENETIC COEFFICIENT IS EVIDENT, "*," THE ORGAN IN WHICH IT CAN PROBABLY BE MEASURED MOST EFFICIENTLY

Genetic coefficients	Vegetative phase		Reproductive phase									Other coefficients of which this may be a further expression
							corolla					
	Axis	Leaf	Axis	Bract	Pedicel	Calyx	Tube	Throat	Limb	Stamen	Pistil	
(1) Cell size	x	x	x	x	x	x	x	*	x	x	x	(2)
(2) Cell elongation	x	x	x	x		x	*		x		x	
(3) Geotropic response	x	x	x	x	*			?	?			(2)
(4) Leaf-vein angles		x							?			
(5) Plastid color		x			x		x	x	*			
(6) Foliar periphery		x		x		x			*		x	
(7) Foliar base		x		x		x			*			(2)
(8) Pollen color										*		
(9) Time of blooming							x	x	*			(5)
(10) Scent							*					
(11) Inflorescence			*									(2)

A tabular summary of the coefficients which we have been able to detect so far is given in table 1. It will be seen that eleven different coefficients have been recognized. Further work may possibly add a few more and will probably reduce certain of those listed as separate to a common coefficient. While there may well be differences which are not accounted for by the action of these eleven, they are certainly responsible for most of the total hiatus between the two species.

In this particular problem, as stated above, an estimate of the coefficients was desired as an aid in the genetic analysis. It may be well, however, by way of example, to point out how the estimate might have been used had our concern been the measurement of differences in populations involving the two species. Only two of the coefficients would be difficult to score, (9) and (10). The effects of both of these coefficients are greatly influenced by environmental factors, and it is also difficult to record them objectively. Of the remaining nine, one, (8), is seemingly manifest only in the pollen, and one, (11), only in the branching of the inflorescence. They would obviously have to be measured at those places. Coefficients (1) to (7), however, are all manifest in both the leaf and the flower, and each of the seven is expressed in various other ways. With the above estimate as a guide we should be able to decide where these seven differences might be measured most efficiently.

Were it not for this previous analysis it might have seemed that the leaf is the most promising organ for measurement. It is practically two dimensional, and its characteristics can all be expressed in simple quantitative terms by measuring and counting the veins and the vein angles. The leaf could furthermore be measured on young plants which had not yet reached the reproductive phase. The above analysis demonstrates, however, that the divergence between the two species can much more efficiently be measured in the flower. Though all seven coefficients are expressed in the leaf, its shape is the resultant of four of them, cell size, cell elongation, basal growth, and peripheral growth. Each of these can be determined in the flower with a single measurement, whereas in the leaf the raw

measurements are a complex resultant of all four. Furthermore, nearly all the veins and vein angles would have to be measured and given a thorough statistical treatment before they would be anywhere nearly as useful as the raw data obtained from the flower. The complexities of integrating and interpreting leaf measurements are illustrated in the statistical papers of Czeczott and her associates (Czeczott, '36, Jentys-Szaferowa, '38, Wiśniewski, '32).

The procedure suggested by the above analysis would be much simpler. The seven coefficients could best be measured as follows:

(1) Cell size.—While this is expressed throughout the plant, it can most efficiently be measured in those organs which are not affected by the other coefficients. The diameter of the pedicel or the diameter of the style might perhaps serve but those organs are so small that errors of measurement would be proportionately large. The throat of the corolla (from the insertion of the stamens to the angle marking the limb) is roughly the same proportion in both species (fig. 1, B), its cells seem to be of the same shape, and the limits to be measured are quite definite.

(2) Cell elongation.—This might also be measured in various parts of the plant, or it might even be measured by testing the effect of tissue extracts upon any standardized auxin indicator. The constricted tube of the corolla, however, offers the simplest measurement. In *N. Langsdorffii* it is less than half a cm. long. In *N. alata* it is 6 to 9 cm. While a small portion of this difference is due to (1), the difference in cell size, it is so slight as to be almost negligible by comparison. One measurement on the tube therefore is an almost perfect reflection of the basic difference in cell elongation between the two species.

(3) Geotropic response.—The angle of inclination made by the leaf, the branches of the inflorescence, or the pedicel of the flower might be measured. There is considerable variation among the leaves, however, depending upon the age of the plant, the time of day, the health of the plant, the position with

relation to the rosette, etc. A more comparable measure of (3) can be made by recording the angle made by the pedicel at the time of anthesis.

(4) Leaf-vein angles.—These are easiest to measure on the largest leaves. The best record we have been able to work out is the angle of the first vein above the petiolar portion of the leaf, on the first or second leaf above the rosette (these leaves are often injured, and more consistent results are obtained by choosing arbitrarily the most symmetrically developed of the two).

(5) Plastid color.—While this difference can be seen along the petiole and on the pedicel, particularly in old specimens, it is much more dramatic in the flower. It is there most readily scored on the inside of the flower. As has been previously reported (Anderson, '39), it is easy to recognize three grades of plastid color in the hybrids.

(6) Foliar periphery.—According to the hypothesis suggested above this coefficient accounts for differences in the leaf margin and the floral margin. It would be difficult or impossible to score in the leaf. In the flower it is one of the coefficients responsible for the difference in the width of the limb. The best measurement we have been able to develop so far is the maximum length of the largest corolla-lobe from its tip to the junction with the throat of the tube. This is probably also conditioned by differences in cell elongation and cell size so that a more direct measurement would be preferable.

(7) Foliar base.—Until the operation of the coefficient has been more definitely worked out it is difficult to decide where it might best be measured. For the present we are using the ratio previously adopted (Anderson, '39) for the lobing index (maximum lobe/adjacent sinus).

In the light of our present knowledge the most efficient measure of the divergence between these two species would be based upon the following, as shown in table 1: length of corolla-throat, length of corolla-tube, angle between pedicel and axis,

color of corolla, length of corolla-lobe, width of corolla-limb to the sinus, angle of basal leaf vein in first leaf above rosette, color of pollen. It will be noted (table 1) that all but one of these can be determined by a single measurement or notation. The original data should then be variously weighted and combined, depending upon the nature of the problem and the use to which the index of specific difference is to be put. Pollen-color and corolla-color differences, for instance, seem to be based on comparatively few genes. In an index designed to be roughly proportional to genic differences, they would be given less weight than measures such as tube length, which are apparently based upon a large number of genes.

It is an interesting fact that, though most of the eleven coefficients are expressed in various parts of the plant, all but one of them are most efficiently measured in the flower. Systematists for two hundred years have emphasized the importance of the flower (and its resulting fruit) in studying relationships between species, genera, families, and orders. It would seem probable that the condition found in these two species of *Nicotiana* must be general among the flowering plants. For reasons whose ontogenetical basis is as yet unknown the germ-plasms of the Angiosperms exhibit their characteristics more conspicuously in the reproductive than in the vegetative phase.

DISCUSSION

A method for the analysis of specific differences through the determination of their genetic coefficients has been developed as a general formula and illustrated by example. Its possible applications are in such different fields that it may be well to indicate three types of problems in which it might be used.

(1) The efficient measurement of specific and subspecific divergence.—The study of evolution by an analysis of variation within and between races and species is older than formal genetics. Until very recently the work of this school has been based on the assumption that if only enough measurements were made and studied with refined mathematical methods,

significant results would emerge. In other words, it was tacitly assumed that organisms vary at random. In our opinion this is putting the cart before the horse. How to measure a specific difference is a research problem which must be undertaken before one takes up the further problem of measuring that difference. As Fisher ('36a) has recently said in discussing the science of craniometry:

> It seems, indeed, undoubtedly true that the theoretical concepts developed . . . have lagged far behind the mass of observational material which has been accumulated. This may be partly due to the sheer magnitude of the programme which the energy of its founders sketched out, partly to an intuitive confidence, widely held in other fields, though everywhere difficult to justify, that, by amassing sufficient statistical material, all difficulties may ultimately be overcome.

The problem of working out even the barest estimate of the genetic coefficients which differentiate the races of men will certainly be much more difficult than the corresponding problem with which we are concerned in *Nicotiana*. Our experience in that latter seemingly unrelated field furnishes a number of suggestions. Biometric study of the races of men has been concentrated upon the skull though our experience with *Nicotiana* suggests that the form of the skull, like that of the leaf, is a complex resultant of many coefficients. It is therefore the worst kind of material for distinguishing between races, since even if there were a clear-cut difference in the basic coefficients separating the races, this would be obscured in its effect on the skull. There seem to be coefficients, for instance, which affect the long bones of the arm and leg in a fairly transparent fashion but cause complex changes in the skull and can be measured there only in an indirect and laborious way. Determinations of variation within and between the races of mankind would yield more significant results if they were based upon records of as many apparently unrelated characters as possible; hair color, hair texture, hair distribution, length of long bones, width of lip, shape of finger nails, finger-print patterns, eye color, and skin color, for instance. An object is much better defined when we describe its weight, color, size, texture, shape, and color pattern than when we have numerous careful

determinations of its weight alone. The latter has until recently been the method of the biometricians.

(2) The genetic analysis of differences between species.—One of the chief sources of evidence for evolutionary changes in the germ-plasm comes from the examination of hybrids between related species. Unfortunately nearly all the evidence which has been accumulated relates to characters rather than to genetic coefficients. To understand what the germ-plasm is doing in a species cross we need to have at least an estimate of the total difference between the parental species and data as to how that total difference is behaving in F_1, F_2, and backcrosses. In most of the published data only one or two obvious differences are followed in this fashion, and even with them the data are reported in terms of such characters as leaf length or plant height. As we have shown above, these characters are the resultants of a number of factors in which the action of any one is very much obscured. If the study of species hybrids could be preceded by at least a rough estimate of the main genetic coefficients which distinguish the parental species, we would have much more direct and dynamic evidence as to differences between related germ-plasms.

(3) The determination of phylogenetic patterns.—If an analysis similar to the one made above could be made for a group of related species it would provide unique data on evolution. While the attempt to consider all the differences between a group of related species in terms of their fundamental coefficients would admittedly be difficult it should not be impossible. Experience with a number of closely related species in several different genera has convinced us that such coefficients as those suggested above operate quite generally among the flowering plants. In *Iris, Acer,* and *Uvularia* closely related species have been found to differ by such general tendencies as absolute cell size, variation in cell size, amount of secondary thickening in cell walls, and geotropic orientation of branches of the axis and of the appendages (Anderson and Hubricht, unpublished). Such a study could most easily be

undertaken in a genus such as *Nicotiana* in which both the leaves and flowers are large and clearly differentiated into definite tubes, limbs, petioles, etc. While it would have to be frankly provisional it would provide a view of phylogeny which would be dynamic rather than static.

SUMMARY

1. From previous studies of closely related species it had been concluded that differences between such species are to be sought not in any one character but in harmoniously integrated tendencies (genetic coefficients) expressed more or less throughout the entire organism. A simple mathematical notation is developed for expressing the resulting morphological hiatus between two species.

2. By way of example, an estimate is made of the genetic coefficients which differentiate *Nicotiana alata* from *N. Langsdorffii*. Eleven such coefficients are suggested, the most important of which affect cell size, plastid development, and the auxin mechanism.

3. Estimates of genetic coefficients might be used in a number of different fields of biology. Their application to the following three problems is discussed: (1) The efficient measurement of specific and subspecific divergence; (2) The genetic analysis of differences between species; (3) The determination of phylogenetic patterns.

BIBLIOGRAPHY

Anastasia, G. E. ('14). Araldica Nicotianae. R. Ist. Sper. Scafati, Boll. Tec. Colt. Tabacchi **13**: 51–220.

Anderson, Edgar ('36a). The species problem in Iris. Ann. Mo. Bot. Gard. **23**: 457–509.

————, ('36b). Hybridization in American Tradescantias. *Ibid.*: 511–525.

————, ('39). Recombination in species crosses. Genetics **24**: 668–698.

————, and Leslie Hubricht ('38). The American sugar maples. I. Phylogenetic relationships, as deduced from a study of leaf variation. Bot. Gaz. **100**: 312–323.

————, and Thomas W. Whitaker ('34). Speciation in Uvularia. Jour. Arn. Arb. **15**: 28–42.

Avery, Priscilla ('38). Cytogenetic evidences of Nicotiana phylesis in the alata-group. Univ. Calif. Publ. Bot. **18**: 153–194.

Brieger, F. G. ('35). Genetic analysis of the cross between the self-fertile *Nicotiana Langsdorffii* and the self-sterile *N. Sanderae*. Jour. Genetics **30**: 79–100.

Czeczott, Hanna ('36). A study on the variability of the leaves of beeches: *F. orientalis* Lipsky, *F. silvatica* L., and intermediate forms. Part II, pp. 1–68 (Polish, English Summary). Reprint from Ann. Soc. Dendrol. Pologne **6**.

Dolk, H. E. ('36). Geotropism and the growth substance. Rec. Trav. Bot. Néerl. **33**: 509–585.

East, E. M. ('16). Inheritance in crosses between *Nicotiana Langsdorffii* and *Nicotiana alata*. Genetics **1**: 311–333.

Fisher, R. A. ('36a). "The coefficient of racial likeness" and the future of craniometry. Roy. Anthrop. Inst. Jour. **66**: 57–63.

————, ('36b). The use of multiple measurements in taxonomic problems. Ann. Eugen. **7**: 179–188.

Jentys-Szaferowa, Janina ('38). Biometrical studies on the collective species *Betula alba* L. II. The possibility of hybridization between species *Betula verrucosa* Ehrh. and *Betula pubescens* Ehrh. (Polish, English translation). Inst. Rech. Forêts. Dom. Warszawa, Ser. A, No. 40, pp. 1–84.

Nagel, Lillian ('39). Morphogenetic differences between *Nicotiana alata* and *Nicotiana Langsdorffii* as indicated by their response to indoleacetic acid. Ann. Mo. Bot. Gard. **26**: 349–374. 1939.

Pearson, Karl ('26). On the coefficient of racial likeness. Biometrika **18**: 105–117.

Sachs-Skalinska, M. ('21). Recherches sur les hybrides du Nicotiana. Mém. Inst. Génét. de l'École Sup. d'Agri. à Varsovie **1**: 47–122.

Smith, Harold H. ('37). Inheritance of corolla color in the cross *Nicotiana Langsdorffii* by *N. Sanderae*. Genetics **22**: 347–360. The relation between genes affecting size and color in certain species of Nicotiana. *Ibid.*: 361–375.

Wiśniewski, Tadeusz ('32). Biometrische Untersuchungen über die Variabilität der Rotbuche *(Fagus silvatica)* in Polen I, pp. 1–27 (Polish, German summary). Reprint from Sylwan **6**: 7–8.

Explanation of Plate

PLATE 24

A. Flower of *Nicotiana alata* (× 7/10).
B. Flower of *N. Langsdorffii* (× 7/10).
C. Same, with calyx removed.
D. Ripe, opened capsule of *N. alata* (× 2).
E. Capsule of *N. Langsdorffii* (× 2).
F. Seed of *N. alata* (× about 50).
G. Seed of *N. Langsdorffii* (× about 50).