The inheritance of certain Quantitative Characters in Tobacco.

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The discovery of NILSSON-EHLE (:09) that certain hybrids are heterozygous for two or more separately inherited factors, either of which is alone capable of producing the same character, laid the foundation for the proof of the generality of the Mendelian interpretation of inheritance in sexual reproduction. NILSSON-EHLE showed that in such cases, with complete dominance, the F_2 ratios became 15:1, 63:1 and so on instead of the simple 3:1 usually obtained.

It was from similar facts obtained independently that EAST (:10) made the first Mendelian interpretation of the inheritance of quantitative characters by assuming absence of dominance, but the few data he presented in its support would not have been convincing if it had not been for the work of the Swedish investigator.

Soon a number of biologists made use of this new development of Mendelism, and the following investigations in quantitative characters may be so interpreted: EAST (:11) and EAST and HAYES (:11) working with height of plants, length of ears, weight of seeds, and row classes of maize, SHULL (:10) row classes of maize, EMERSON (:10) for shapes and sizes in maize, beans and gourds, TAMMES (:11) for certain characters of *Linum* forms, TSCHERMAK (:11) (:12) for time of flowering in peas and for weight of seeds, HAYES (:12) for height of plants, area of leaves, and number of leaves in tobacco, PHILLIPS (:12) for body size in ducks, DAVIS (:12) for *Oenothera* characters, WEBBER (:12) for plant characters of peppers, GILBERT (:12) for plant characters in tomatoes, BELLING (:12) for certain characters of beans, McLENDON (:12) for cotton characters.

The difficulty of determining the inheritance of such complex characters correctly is greatly increased, owing to the fact that

environment plays such a large part in their development. Fluctuating variability is always high, yet many careful investigations have proved that such fluctuations are not inherited. Such fluctuations greatly interfere with the analysis of the inheritance of size characters, as it is impossible except by a breeding test to determine whether a certain deviation from the mean is of a heritable nature or is simply due to environmental conditions. Fluctuating variability as used here refers to the somatic variations which are non-inherited, and not to germinal variations which are inherited.

The only way to determine the inheritance of quantitative characters is to compare the variability of the F_1 and F_2 generations and the parents, growing all under as nearly similar environmental conditions as possible. The variability of the F_1 generation should be no greater than that of the parents, and the F_2 generation should give a greatly increased variability. When sufficient numbers are studied, the F_2 generation should show a range of variation equal to the combined range of the parents and F_1 . Certain F_2 forms should breed true in F_3 , giving no greater variability than the parent types; other F_2 forms should give decreased variability in F_3 when compared with the F_2 generations; and others should show as great variability as the F_2 generation itself.

In most of the above investigations no F_3 generations have been reported, and where reported they comprise only a few forms. In an earlier paper, data were given for the parental types, also for the F_1 and F_2 generations of tobacco crosses, and the inheritance of such characters as leaf number, size of leaf and height of plants was interpreted as due to the interaction of several independently inherited factors, the heterozygous condition of each factor being half the homozygous. The purpose of this paper is to give further data on the inheritance of leaf number in tobacco, of which a number of F_3 generations have been grown.

The Methods Used.

The parents of the crosses reported here, with the exception of the Broadleaf, were strains which had been inbred for a number of years and which were therefore of uniform type. Unguarded fertilized seed of the Broadleaf plants used for crossing has proved very uniform, so we are justified in concluding that this cross was also made between pure parents.

Different generations of the parent types have been grown to compare with the crosses and also to give an idea of the variability of the parents.

Fig. 1. 403. Sumatra. Averages about 27 leaves per plant.

The parents have each been given a number; as Sumatra, 403, Broadleaf, 401. A cross between them has been designated as 403×401 , the female parent appearing first. Later generations have been grown as $(403 \times 401)-1$, $(403 \times 401)-2$ etc.

The counts of leaf number with which this article deals were made from the fourth leaf from the bottom to the leaf below the bald sucker at the top. This represents the number of leaves usually

harvested and allows of topping plants by the usual farm practice, with the exception of those saved for seed.

It has been shown in a previous article, HAYES (:12), that the number of leaves per plant is a character which is little affected by environment. Thus, the mean for each of four inbred types grown at Forest Hills, Mass., on a well fertilized soil, at Bloomfield, Conn., on a heavily fertilized typical tobacco soil, and at New Haven, Conn., on a very light gravelly soil only moderately dressed with fertilizers, gave a variation from the average mean of the type of $\pm .8$ leaves per plant.

Data on two crosses are given in this article, but for convenience each cross will be separately discussed.

Family (403 $\times$ 401) Sumatra $\times$ Broadleaf.

The Sumatra parent had been grown under shade in Connecticut from seed inbred for several generations.

The Broadleaf parent has been grown in the open in Connecticut since the early history of the tobacco industry and is of a very uniform type.

The cross was made in 1909 and in 1910 the parent types and F_1 were grown in New Haven. Inbred seed of the parents and F_1 were grown in New Haven in 1911 and several large cultures of the F_2 generation were also grown in Bloomfield. In 1912 a later generation of the parents and five F_3 generations from selected F_2 plants were grown in New Haven. Eight F_3 generations were also grown in Bloomfield.

In Table 1 from left to right in separate columns are given the selection numbers, the year grown, the leaves of the parent plant when known, the range of variation, the number of plants counted, the mean value for number of leaves, and the coefficient of variability, the two latter calculations being those of greatest value.

There seems to be a gradual decrease for leaf number, and in 1912 an increase of variability for No. 403, Sumatra.

As the results of a count of leaf number for 1911 and 1912 are very similar being $26.5 \pm .11$ and $26.2 \pm .12$ respectively, it may be possible that the mean in 1910 which was $28.2 \pm .08$ is somewhat too high due to experimental errors or to a deeper setting of the parent plants. The reason for the great increase in the Coefficient of variability in 1912 is harder to explain. A manure heap lay in the fall of 1911 on one end of the row on which this selection grew

in 1912 while the remainder of the row was only moderately fertilized. The plants on the rich end grew very rankly and gave a range in variation from 22 to 32 leaves while the remainder of the row varied from 21 to 30 leaves. Another variety gave as high counts for leaf number when the plants were grown in 4 inch pots as in the open field under favorable conditions however, it is possible that the Sumatra variety is more susceptible to unfavorable conditions than some other types. The matter is discussed at this point as the

Fig. 2. 401, Broadleaf. Averages about 19 leaves per plant.

Coefficient of variability ($8.28 \pm .32$) of 403-1-1 is higher than should be obtained in an inbred line under favorable conditions.

Taking these points into consideration this Table shows the following facts: The F_1 generation is intermediate for leaf number, the Sumatra parent for 1910 giving a mean production of $28.2 \pm .08$ leaves; the Broadleaf, $19.2 \pm .05$; and the F_1 generation $23.6 \pm .07$.

The variability of the F_1 generation and the parents was about the same. The Coefficient of Variability of the Broadleaf parent for

1910 was $5.00 \pm .19$, of the Sumatra $5.27 \pm .21$, and of the F_1 $5.51 \pm .21$.

Seeds from three F_2 plants bearing 21, 24 and 25 leaves respectively were grown in Bloomfield in 1911, large cultures of each being planted. Progeny of two of these plants were also grown in New Haven and as they gave similar results space is not taken in the

Fig. 3. (403 × 401)—1—4, Sumatra × Broadleaf, F_3 .

This selection bred true for the drooping leaf habit of the Broadleaf, an intermediate leaf size and an average production of 22 leaves per plant.

Table for them. These F_2 generations gave a range of variation equal to the combined range of the parents and F_1 and a corresponding increase in the coefficient of variability. All gave similar results showing the variation of the F_1 generation to be noninherited.

The most interesting facts shown by the Table are the means and coefficients of variability for number of leaves of the thirteen

F₃ generations. Of these thirteen selections, two show about the same variability as the parents, four are intermediate in variability between the parents and F₂, three show about the same variation as the F₂ generation, and four show a slightly larger variability than the F₂ generation. Thus the coefficient of variability in F₃ ranged from $6.44 \pm .27$ in (403 $\times$ 401)—1—2 to $10.84 \pm .39$ in (403 $\times$ 401)—1—13.

It is of interest to note that of the two types which were as uniform as the parents, that both were of intermediate value, as compared with the parents for leaf number, one giving a mean for leaf number of $22.5 \pm .09$ and the other giving a mean of $23.9 \pm .08$ leaves.

The means of the F₃ generations were of different values, ranging from $20.4 \pm .08$ in No. (403 $\times$ 401)—2—1 to $26.3 \pm .20$ in selection No. (403 $\times$ 401)—1—8.

Table 1.

Number of Leaves of Sumatra, 403, Broadleaf, 401, and F₁, F₂, and F₃ generations of a Cross between 403 and 401.

No.	Year Grown	Leaves of Parents	Range of Variation	Total	A.	C. V.
403, Sumatra	1910	—	24—31	150	$28.2 \pm .08$	$5.27 \pm .21$
403—1	1911	29	23—31	125	$26.5 \pm .11$	$6.64 \pm .28$
403—1—1	1912	29	21—32	151	$26.2 \pm .12$	$8.28 \pm .32$
401, Broadleaf	1910	—	17—22	150	$19.2 \pm .12$	$5.00 \pm .19$
401—1	1911	20	16—22	108	$19.1 \pm .05$	$6.54 \pm .30$
401—1—1	1912	22	17—23	145	$19.9 \pm .07$	$6.08 \pm .24$
(403 $\times$ 401) F ₁	1910	—	19—26	150	$23.6 \pm .07$	$5.51 \pm .21$
(403 $\times$ 401)—1 F ₂	1911	25	17—32	2402	$22.8 \pm .03$	$9.52 \pm .11$
(403 $\times$ 401)—3 F ₂	1911	24	17—35	1632	$22.7 \pm .03$	$8.99 \pm .11$
(403 $\times$ 401)—4 F ₂	1911	21	17—33	1958	$22.5 \pm .03$	$9.51 \pm .10$
(403 $\times$ 401)—1—2 F ₃ ...	1912	25	19—29	131	$22.5 \pm .09$	$6.44 \pm .27$
(403 $\times$ 401)—1—3 F ₃ ...	1912	26	17—29	186	$24.1 \pm .10$	$8.51 \pm .30$
(403 $\times$ 401)—1—4 F ₃ ...	1912	24	18—26	182	$22.0 \pm .08$	$7.54 \pm .27$
(403 $\times$ 401)—1—6 F ₃ ...	1912	23	20—28	194	$23.9 \pm .08$	$6.61 \pm .23$
(403 $\times$ 401)—2—1 F ₃ ...	1912	21	15—25	188	$20.4 \pm .08$	$8.04 \pm .24$
(403 $\times$ 401)—1—7 F ₃ ...	1912	22	17—28	207	$21.5 \pm .10$	$10.14 \pm .34$
(403 $\times$ 401)—1—10 F ₃ ..	1912	26	19—27	151	$23.5 \pm .10$	$7.83 \pm .30$
(403 $\times$ 401)—1—12 F ₃ ..	1912	25	18—30	209	$23.7 \pm .14$	$10.51 \pm .41$
(403 $\times$ 401)—1—8 F ₃ ...	1912	28	19—33	82	$26.3 \pm .20$	$10.38 \pm .55$
(403 $\times$ 401)—3—5 F ₃ ...	1912	27	17—28	159	$21.7 \pm .11$	$9.45 \pm .36$
(403 $\times$ 401)—3—6 F ₃ ...	1912	28	16—27	229	$22.5 \pm .09$	$8.71 \pm .26$
(403 $\times$ 401)—3—8 F ₃ ...	1912	25	17—23	85	$20.6 \pm .12$	$8.25 \pm .43$
(403 $\times$ 401)—1—13 F ₃ ..	1912	25	16—29	179	$22.5 \pm .09$	$10.84 \pm .39$

Family (402 $\times$ 405) Cuban $\times$ Havana.

The Cuban variety No. 402 is the type now grown so widely under shade in the Connecticut valley. It was produced by selection from imported Cuban seed and at the time the cross was made had been inbred for a number of years.

Fig. 4. 402—I—I, Havana, Grown under shade at Bloomfield, 1912.

The Havana parent, No. 405, has been grown in Connecticut for a long term of years and at the time the cross was made had been inbred two years.

This cross was made in 1909, the parents and F_1 being grown at Bloomfield in 1910, the F_2 and parents in 1911, and the F_3 and parents in 1912, all being grown under shade.

Table 2 gives results for this cross similar to those shown in Table 1 for the Sumatra $\times$ Broadleaf cross. This table shows that

the parents and F_1 average about the same number of leaves, the F_1 generation having about the same variability as the parents.

The F_2 generation varied greatly, giving a coefficient of variability of $15.84 \pm .54$. Plants were produced in this generation with a much higher and a lower number of leaves than in F_1 , the range of variation being from 14 to 33.

Five F_3 generations were grown from selected F_2 plants bearing 20, 20, 22, 28 and 30 leaves respectively. One of the F_3 generations gave a range of variation from 15 to 22 leaves, a mean value of $18.4 \pm .09$ and a coefficient of variability of $8.56 \pm .34$, thus showing only a little greater variation than the parent types. Two gave variabilities as great as in F_2 , while those grown from 28 and 30 leaved F_2 parent plants respectively gave intermediate variabilities between F_1 and F_2 and mean values of $26.6 \pm .16$ and $28.8 \pm .28$ leaves per plant.

Thus, from crossing two strains having mean values of about 20 leaves per plant a new type has been produced with a larger number of leaves.

Table 2.

Number of Leaves of Havana, 402, Cuban, 405, and F_1 , F_2 , and F_3 generations of a Cross between 402 and 405.

No.	Year Grown	Leaves of Parents	Range of Variation	Total	A.	C. V.
405, Cuban	1910	—	16—25	150	$19.9 \pm .08$	$7.53 \pm .28$
405—I	1911	21	18—23	124	$20.6 \pm .07$	$5.29 \pm .23$
405—I—I	1912	23	17—25	150	$20.9 \pm .07$	$6.17 \pm .24$
402, Havana.....	1910	—	17—24	150	$19.8 \pm .07$	$6.98 \pm .27$
402—I	1911	20	16—25	143	$20.3 \pm .10$	$8.87 \pm .35$
402—I—I	1912	20	17—22	150	$19.4 \pm .05$	$4.59 \pm .18$
(402 $\times$ 405) F_1	1910	—	15—25	150	$19.8 \pm .07$	$6.10 \pm .24$
(402 $\times$ 405)—I F_2	1911	—	14—33	192	$20.9 \pm .16$	$15.84 \pm .54$
(402 $\times$ 405)—I—2 F_3 ...	1912	20	13—29	112	$19.7 \pm .18$	$14.67 \pm .67$
(402 $\times$ 405)—I—3 F_3 ...	1912	20	15—22	142	$18.4 \pm .09$	$8.56 \pm .34$
(402 $\times$ 405)—I—4 F_3 ...	1912	28	20—35	148	$26.6 \pm .16$	$11.20 \pm .44$
(402 $\times$ 405)—I—5 F_3 ...	1912	30	22—34	45	$28.8 \pm .28$	$10.00 \pm .20$
(402 $\times$ 405)—I—6 F_3 ...	1912	22	13—29	201	$20.1 \pm .15$	$16.17 \pm .56$

These two types, Cuban and Havana, differ in leaf size, the Havana giving a somewhat larger leaf area. Both types have about the same leaf width but the Havana leaf is longer. The F_1 generation was of intermediate leaf size and in F_2 there was an increase

of variability. Among the F_3 generations grown (402 $\times$ 405)—1—2 was selected because it resembled Cuban. Although no statistical results can be given here we may say that this selection bred true

Fig. 5. (402 $\times$ 405)—1—3. A representative plant of an F_3 generation of a cross between Havana and Cuban. This selection bred true for the Havana type of leaf.

for the Cuban leaf shape. The parent plant of the selection (402 $\times$ 405)—1—3 closely resembled Havana in leaf shape and, as we have already noted, bred comparatively true for leaf number. This selection bred true for the Havana type of leaf.

Summary of Results.

1. Number of leaves per plant is a stable character and little affected by changes of environment.

2. The F_1 generation is of intermediate value, the mean for leaf number being almost exactly equal to the average of the parental means.

3. The F_1 generation is no more variable than the parents.

Fig. 6. 405—1—1, Cuban. Grown under shade at Bloomfield, 1912.

4. Different variates in F_1 give similar results in F_2 showing the F_1 variation to be of no germinal value.

5. The F_2 generation is much more variable than the parents or F_1 .

6. Some of the F_2 generation breed true in F_3 , giving no greater variability than the parents themselves, others give an intermediate variability between that of the parents and F_2 , and others are as variable as the F_2 generation itself.

7. Intermediates as well as extremes may breed true. Of the three F_3 generations which bred true for leaf number, giving no greater variation than the parents, two were intermediates.

Interpretation of Results.

The above results are a statement of the behavior of parents and crosses under careful observation.

These results in the light of the discovery of multiple factors for qualitative inheritance seem most easily and helpfully interpreted as due to the interaction of several independently inherited units or genes. Each of these independent interchangeable units, or genes, allelomorphous to its own absence, is capable of adding to the character, and the heterozygous condition of any unit is half the homozygous.

Let us apply this to our tobacco crosses. Suppose, for example, in cross (403×401) that each of our parents is pure for the same basal condition of 20 leaves and that in addition the Sumatra parent, No. 403, has some inherited properties which result in a production of 26 leaves. Let us suppose this is due to three interchangeable allelomorphous character pairs, each inherited separately, and that the heterozygous condition is half the homozygous condition.

If we follow the usual Mendelian method and represent our characters by A, B and C and their absence by a, b and c, we get a condition in F_1 of AaBbCc or 23 leaves. In F_2 we will get a range of germinal variation of from 20 to 26 leaves.

In a total of 64 F_2 individuals, 8 will breed true in F_3 , and of these eight 1 will breed true for each parent form or for 20 and 26 leaves, 3 will breed for 22 leaves, and 3 for 24 leaves. Of the remaining 56, 8 will give as great variability as the F_2 generation, 24 will give a variation for one character pair, and 24 for two character pairs.

Of the 13 F_3 generations of cross (403×401), Table 2, two gave no greater variation than the parents, i. e., on the average, 1 in $6\frac{1}{2}$ bred true, while the expectation, if three allelomorphous pairs are involved is that 1 in 8 will breed true in F_3 .

Considering now our cross (401×405), in which both parents averaged about 20 leaves per plant, we find a great increase of variability in F_2 . This condition is very easily explained by our hypothesis. If we suppose the parent forms to be represented by gametic values for leaf number of 16 AABb and 16 CCDD we will obtain in F_1 16 AaBbCcDd, or 20 leaves, and in F_2 a greatly increased variability.

Of the five F_3 generations of cross (402 $\times$ 405) which were grown, one was somewhat more variable than the parents, two gave a variation about intermediate between F_1 and F_2 , and two were as variable the F_2 generation itself.

Fig. 7. (402 $\times$ 405)—1—2. A representative plant of an F_3 generation of a cross between Havana and Cuban. This selection bred true for the Cuban leaf shape.

The most interesting feature of the cross is the fact that of the F_3 generations, those two which gave an intermediate variability had a much larger mean than that of either parent. According to our hypothesis, 16 AABBCDD should give a type breeding true for 24 leaves. These assumptions illustrate the idea although in the actual cross the conditions are more complex, due undoubtedly to the interaction of a greater number of factors.

That two out of the five F_3 generations of this cross bred true to the parental leaf shape is a further reason for a belief in a Mendelian inheritance of quantitative characters.

Fig. 8. From left to right middle leaves of Plants shown in Figures 4—7. At the left, (402 X 405)—1—3, next 402—1—1, Havana, then 405—1—1, Cuban and at the right, 402 405)—1—2. Grown under cloth shade ad Bloomfield, 1912.

Conclusion.

Our results show the F_1 generation of a cross between pure lines of tobacco to be of a uniform nature and no more variable than the parents. Different F_1 individuals gave similar results in F_2 , showing the variability in F_1 to be of no germinal value. In F_2 there was an increase of variability and, where a sufficient number of individuals were grown, there was a range of variation equal to the combined range of variation in the parents. Certain F_2 individuals bred true in F_3 , giving no greater variability than the parent forms, while others gave variabilities ranging from the parents to that the F_2 generation.

These results give further confirmation of the hypothesis that the inheritance of quantitative characters, such as size, shape and number of various plant organs may be due to the interaction of a multiplicity of factors, each inherited separately and capable of adding to the character, the heterozygous condition being half the homozygous. The difficulty of determining the number of factors involved is very great, owing to the obscuring effect of fluctuating variability.

Whatever may be the ultimate accepted hypothesis concerning inheritance of quantitative characters, from the plant breeding stand-

point, the practical manipulation of them does not seem to the writer to be greatly different than that of qualitative characters. In both it is an undoubted fact that one may obtain new combinations of the parental characters ranging from the value of one parent to that of the other; in both some of these F_2 forms will breed true, while others will continue to recombine the factors in which they are heterozygous.

In conclusion the writer desires to express, his obligation to DR. E. M. EAST for much helpful advice.

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