INHERITANCE IN NICOTIANA TABACUM. XVIII. MONOSOMIC ANALYSIS

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THE FIRST accounts of monosomics in *Nicotiana Tabacum* (CLAUSEN and GOODSPEED 1926a, b) reported establishment of two types and demonstrated that one of them, haplo-C (then called "corrugated"), involved the chromosome in which the basic color factor, *Wh*, is located. These results were the beginning of a program designed to establish as many monosomic types in *Tabacum* as possible and to take advantage of their favorable features for analytical studies of Mendelian characters. The investigations have now progressed to a successful isolation of 24 different monosomics which are believed to represent a complete set of the primary types.

THE PRODUCTION AND MAINTENANCE OF MONOSOMIC STOCKS

Of the first two monosomics, haplo-F appeared as a spontaneous variant in a normal progeny and haplo-C was isolated as a derivative type from a cross between *Tabacum* and *sylvestris*. OLMO (1935) has described how monosomics may be isolated by design from crosses between *Tabacum* and *sylvestris* or *tomentosa* followed by recurrent backcrossing to *Tabacum*. Theoretically this method should lead to isolation of all possible monosomic types; in practice, however, the considerable heterozygosity set up in these crosses has been found to confuse the recognition and establishment of types. Nevertheless, a number of monosomics have been secured by this method, and recurrent backcrossing eventually does eliminate the troublesome segregation due to heterozygosity.

In more recent years, the asynaptic *Tabacum* type, pale-sterile, performs a similar service without the deterrent features of gene segregation, since pale-sterile has the same residual genetic constitution as the normal type. Pale-sterile has, on the average only 11 bivalents per microsporocyte, the rest of the chromosomes remaining unassociated. The progenies obtained from crosses of pale-sterile ♀ × normal ♂ consist of plants of marked diversity, as a consequence of random distribution of unassociated chromosomes. The variation is so considerable that it is difficult to find two plants exactly alike among the offspring. Aside from an occasional triploid, the offspring consists of unbalanced diploids. Relatively few, however, are simple monosomics or trisomics; most of them are more complex—double or triple monosomics, monosomic trisomic combinations, etc. These complex types, however, may readily be reduced to their elementary components by further crosses to normal.

The use of pale-sterile has another advantageous feature—namely, that in certain circumstances it permits isolation of specific monosomics by design. As an example, a cross of pale-sterile carmine with normal white produces a

small percentage of white offspring, which must perforce be monosomic for the chromosome containing the white locus. Such isolation by design has been practiced in a number of instances; but its utility is unfortunately seriously limited by the dearth of available simple recessive characters in the species.

As a matter of fact, however, the rate of sporadic appearance of new monosomics, especially among progenies of established ones, is so high as to have taxed our resources in studying and testing them. These sporadic variants have been the chief source of the monosomics now in our possession. Doubtless they owe their origin to the mildly non-conjunctional behavior which has been observed in many of the monosomic types.

Whatever the source, the monosomic stocks are maintained by recurrent backcrossing (monosomic ♀ × normal ♂) to the standard *Tabacum* type, Purpurea (UCBG 06-25). This method not only assures identity of the residual genotype in our stocks, but, more important, it also guards against permanent alteration in the constitution of the monosome, which in each generation is derived from the normal male parent.

Despite this method of maintenance, however, there are some modified monosomic types which may be difficult to distinguish from primary, unmodified ones. Two of the twenty-four which are now in our possession—namely, haplo-U and haplo-Z—may be considered somewhat dubious, mainly because they exhibit a relatively high frequency of non-conjunction in microsporocytes. Until more satisfactory tests than those now available can be devised, some question may remain as to the validity of some of our types, but at any rate even the dubious ones are monosomic. Whatever doubt remains resolves into a question of whether or not they are all primary types.

The maintenance of 24 monosomic types naturally enough entails considerable labor and effort in the growth of stocks and the scoring of cultures each year, particularly since many of them have relatively low transmission rates. In later years, however, with more greenhouse space available, we have found it possible to reduce the overhead materially and to maintain a full collection of the types, available at all times for such studies and crosses as may seem desirable, by propagating selected plants from cuttings. *Tabacum* cuttings root readily, particularly when treated with standard rooting agents. This procedure enables us almost indefinitely to maintain plants verified for chromosome number and tested for transmission. In view of the extraordinarily favorable reproductive features of *Tabacum*, it has always been possible to make as many crosses, sometimes a very considerable number, on such greenhouse plants as have been necessary for our purposes.

MORPHOLOGICAL FEATURES

Monosomics differ from the standard type, Purpurea, and from one another in a specific ensemble of morphological features. In this respect they resemble trisomics, although in our experience they are not so readily recognizable as the trisomics of *Datura stramonium* or *Nicotiana sylvestris*, which is understandable, however, in view of the larger number of chromosomes and possibly

also of the dampening effect of the amphidiploid constitution of *Tabacum*. However, judged against a uniform genetic background, as has been done in these investigations, they are all sufficiently distinctive to permit reasonably accurate separation from the normal type on the basis of morphological features, which is all that is necessary for their successful employment. They differ as respects relative ease of recognition; some are very distinctive and may be scored without difficulty even if there is a considerable variation in the genetic background; others require more careful examination, sometimes with resort to auxiliary diagnostic features, presently to be described.

Although all parts of the plant are more or less distinctively affected, in actual practice classification is based chiefly upon flower features, because of their relatively greater stability under variable conditions of growth. It has been found advisable for accurate scoring to measure typical flowers from each plant, not so much for the sake of the measurements themselves as for the necessity it imposes of careful scrutiny of each plant. Many flower features have been found useful for discrimination; among them tube length and limb spread, intensity of coloration, form of the limb and of the corolla lobes, prominence of the infundibulum, style and filament length both absolute and relative, time of dehiscence of the anthers, size and character of the calyx and of the capsule, and numerous other relatively slight, quantitative features, which, although individually insignificant, together may present an ensemble of differences of sufficient magnitude to permit ready recognition. The vegetative features offer auxiliary indicia; particularly size of plant, rate of development, intensity of chlorophyll coloration, leaf shape, and character and degree of development of the auricles. The task of keeping the distinctive features of twenty-four different types in mind is not easy, especially as some of them closely resemble each other; but in any given culture, the necessary discrimination is between the normal and a single specific monosomic type, which materially simplifies classification.

SYLVESTRIS-TABACUM HYBRIDS

When a monosomic *Tabacum* is crossed with *sylvestris* the progeny consists of two classes of hybrids, the normal 36-chromosome *sylvestris-Tabacum* hybrid and a 35-chromosome hybrid which lacks a specific *Tabacum* chromosome. It is, therefore, possible by crossing each of the monosomic types with *sylvestris* to set up a complete series of 35-chromosome hybrids corresponding to the 24 monosomic *Tabacum* types.

The 35-chromosome hybrids are readily distinguishable from the normal hybrid, and usually the morphological features of difference are more pronounced than those of the corresponding monosomic *Tabacum* types. In most cases the features of difference resemble those of the corresponding monosomics, but in a number of instances they include qualitative as well as quantitative differences. The 35-chromosome *sylvestris-Tabacum* counterparts of haplo-C and haplo-G have white instead of colored flowers, that of haplo-F has coral flower color, and that of haplo-P has pink flowers. Haplo-E *Tabacum*

is relatively slightly different from the normal type, but its 35-chromosome *sylvestris-Tabacum* counterpart is very distinct, particularly as to vegetative features. It has strongly bullate, drooping leaves, closely appressed against the main stem. Similar monosomic types may produce distinctly different *sylvestris-Tabacum* counterparts. Thus haplo-F and haplo-N resemble each other closely, but the *sylvestris-Tabacum* counterpart of haplo-F has coral flowers while that of haplo-N has intensely-colored carmine flowers. The 35-chromosome hybrids, therefore, provide an additional means of discrimination which may be extremely useful in resolving questions of doubt.

REPRODUCTIVE FEATURES

The reproductive features of specific monosomics appear to be as distinctive as their morphological features. Some studies have been made of the distinguishing features of the monosome in microsporocyte preparations, of the behavior of the monosome in meiosis, of pollen preparations, of ovular abortion, of seed production, and of transmission ratios. The pertinent quantitative data are collected in table 1.

The monosomes

In good M-I acetocarmine or acetoorcein miscrosporocyte smear preparations, the monosome is easily distinguished from the bivalents, partly by its peripheral position in the plate, partly by its shape and partly by its relative depth of staining. As a consequence it is possible to study the distinctive features of specific chromosomes. While of course the chromosomes are not well suited for revealing minute differences of morphology at this stage, it is possible to determine grosser features of size and shape, as shown by the camera lucida drawings reproduced in figure 1. The figure depicts the differences which occur among the monosomes and something as to the variation which is encountered in dealing with them. Despite the relative inferiority of this stage for studies of morphological features, it is possible to use the cytological preparations for diagnostic purposes much more effectively than would appear feasible from the figure.

Meiotic behavior

Microsporocyte preparations have also been studied in order to determine the frequency and character of deviations from the normal type of association, 23II+1I. Two such types of deviation are outstanding: (1) appearance of trivalents, by association of the monosome with a bivalent—that is, 22II+1III —and (2) non-conjunctional behavior through failure of association of one or more pairs of bivalents to give cells exhibiting 22II+3I, 21II+5I, etc. The former type of behavior occurs in haplo-D and haplo-S, along with non-conjunctional behavior, to the extent of approximately 25 and 20 percent, respectively. In other monosomics trivalent associations are very rare. Non-conjunctional behavior, almost exclusively restricted to a single pair of chromosomes has been noted as occasional in haplo-J and haplo-L, about 5 percent in haplo-

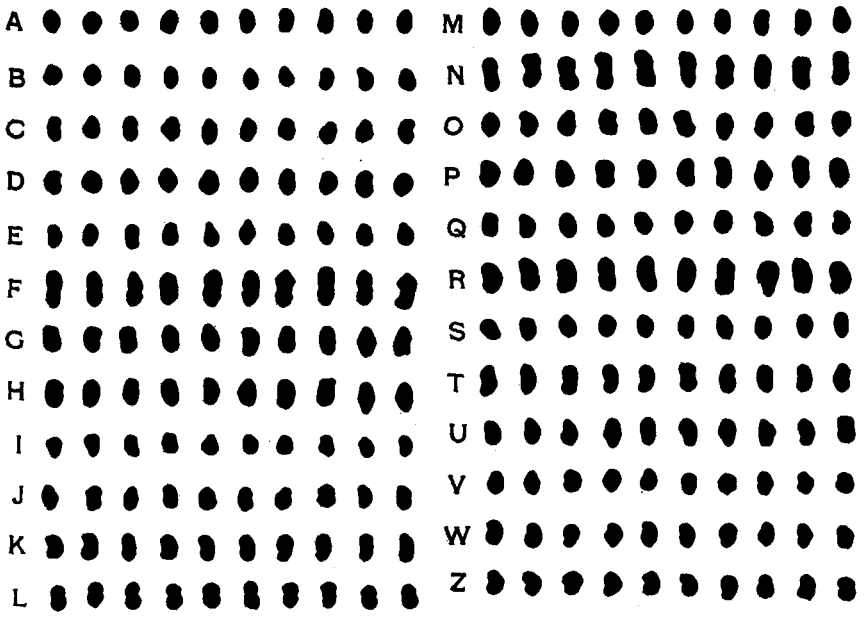

FIG. 1.—The monosomes of *Tabacum*. Ten monosomes of each type as they appear at I–M in microsporocytes.

O, about 10 percent in haplo-H and haplo-I, about 15 percent in haplo-Q, about 25 percent in haplo-U and possibly even more in haplo-Z. In the latter two, non-conjunction may occur simultaneously in two or more pairs of chromosomes, and its occurrence is reflected by production in their progenies of numerous chromosomal variants besides the expected monosomics. No doubt this phenomenon is also responsible for the relatively high sporadic production of exceptional monosomics in other monosomic progenies.

The F chromosome exhibits nucleolar association, but there is at least one other chromosome, as yet unidentified, which is also attached to the nucleolus.

Subgenomic classification of the monosomics

The normal *sylvestris-Tabacum* hybrid, as has been shown by GOODSPEED (1934), exhibits 12II+12I as the modal condition for chromosome association. There are some variations in association, but not one of them approaches the frequency of the modal type which appears in about 60 percent of the microsporocytes. The 35-chromosome hybrids are of two classes: one with a modal association of 12II+11I; the other, 11II+13I; the former indicates that the missing chromosome belongs to the *tomentosa;* the latter, to the *sylvestris* subgenome of *Tabacum*. The fidelity of this behavior confirms the assumption of specificity of association of chromosomes in the hybrid upon which the original hypothesis of the amphidiploid constitution of *Tabacum* was based (CLAUSEN 1928). There are twelve types in each class. For convenience, mono-

somics involving chromosomes of the *tomentosa* subgenome have been given the letter designations A to L, and those of the *sylvestris* subgenome, M to Z. X and Y have been reserved for non-specific characterization of any monosomic or of an unknown type. On the basis of this classification a rough comparison may be made of monosomes of the two groups, by dividing them into the classes indicated below:

	tomentosa subgenome	*sylvestris* subgenome
very small	B I	
small	C E	
medium small	A J K	Q S V W Z
medium	D L	M O
medium large	G H	T U
large	F	N P R

From this grouping it is clear that the members of the *tomentosa* subgenome exhibit a wider range in chromosome size and a smaller average size than those of the *sylvestris* subgenome, but the distinction between the two does not appear to be so great as earlier studies of *tomentosa-Tabacum* and *sylvestris-Tabacum* preparations led us to expect (CLAUSEN 1932).

Pollen conditions

Pollen conditions have been studied in acetocarmine preparations made by thoroughly mixing the entire contents of a single anther in a drop of the stain. Such preparations unfortunately are not permanent, but more lasting ones may be made by mounting the grains in lactophenol lightly stained with acid fuchsin. While these preparations are not so revealing as those stained with acetocarmine, they are useful to keep on hand as pollen type specimens of the monosomics.

The data in table 1 record the number of subnormal grains in the counts. This method of treatment was necessitated by the great variety of conditions exhibited by the preparations and by the difficulty of devising any hard and fast system of classification applicable to all types. In some cases the pollen grains appeared to be sharply divisible into good and aborted grains, the latter completely devoid of contents, or nearly so. Such is the case for those with high abortion rates (around 80 percent), which have been marked with an asterisk. In those with a relatively low rate of pollen abortion (A-B-C-D-E-H-S), classification was more difficult because of a gradation of pollen type which interfered with a purely objective distinction between good and bad grains. In these, only the completely aborted grains were classified as subnormal, although many other grains were obviously defective. Measurements of the good grains of preparations exhibiting high abortion revealed that they were equivalent to those of normal Purpurea; and this equivalence seemed to be complete, both as to measurements and as to condition of the contents. Measurements of good grains of those preparations with a low percentage of

TABLE 1

Reproductive features of the monosomic types of Nicotiana Tabacum.

TYPE	POLLEN ABORTION			OVULE ABORTION			SEED PRODUCTION		OVULAR TRANSMISSION		
	TOTAL	SUB-NORMAL	PER-CENTAGE	TOTAL	ABORTED	PER-CENTAGE	SEEDS PER CAPSULE	PERCENTAGE	TOTAL	MONOSOMIC	PER-CENT AGE
Pp	922	30*	3.3	259	7	2.7	2827	100.0			
A	333	23*	6.9	457	82	17.9	3296	116.6	1291	1016	78.7
B	315	16*	5.1	326	100	30.7	1326	46.9	1303	421	32.3
C	341	83*	24.3	254	82	32.3	2354	83.3	1331	610	45.8
D	469	15*	3.2	263	41	15.6	2093	74.0	835	344	41.2
E	239	16*	6.7	263	77	29.3	1757	62.2	819	671	81.9
F	950	735	77.4	270	66	24.4	1646	58.2	879	526	59.8
G	452	342	75.7	451	380	84.3	535	18.9	972	62	6.4
H	453	101*	22.3	326	148	45.4	2281	80.7	888	625	70.4
I	656	512	78.0	449	345	76.8	536	19.0	520	40	7.7
J	368	297*	80.7	358	311	86.9	181	6.4	432	26	6.0
K	658	523	79.5	471	264	56.1	1135	40.1	381	184	48.3
L	604	478*	79.1	398	290	72.9	534	18.9	183	34	18.6
M	434	362*	83.4	291	95	32.6	945	33.4	803	480	59.8
N	567	446	78.7	346	274	79.2	376	13.3	784	173	22.1
O	529	454	85.8	306	50	16.3	2517	89.0	1085	838	77.2
P	579	446	77.0	326	275	84.4	739	26.1	1049	68	6.5
Q	417	336*	80.6	241	191	79.3	668	23.6	845	93	11.0
R	427	319*	74.7	284	131	46.1	1321	46.7	1074	584	54.4
S	466	58*	12.4	254	54	21.3	2576	91.1	1093	339	31.0
T	747	507*	67.9	316	271	85.8	417	14.8	437	48	11.0
U	414	256*	61.8	322	238	73.9	542	19.2	231	84	36.4
V	497	373*	75.1	665	530	79.7	638	22.6	242	19	7.9
W	538	400*	74.3	546	430	78.8	752	26.6	234	12	5.1
Z	671	551*	82.1	383	316	82.5	164	5.8	223	18	8.1

* Subnormal grains completely aborted, or nearly so.

complete abortion, however, revealed a wider range extending toward lower values than those of normal preparations. Some of the measurements disclosed distinctly bimodal distributions, the mode at the higher value corresponding to that of normal grains, the one at the lower level, marking the larger portion of the distribution, presumably representing the 23-chromosome grains. The remaining preparations were more or less distinctly dimorphic, with the subnormal class consisting of defective grains, distinctive for each monosomic type, marked by various features, such as smaller size, shrunken contents, or

peculiar staining of the contents, while the good ones were again equivalent in every respect to normal grains.

The studies of pollen preparations seem to show clearly that initially the microspores of each monosomic consist of 24- and 23-chromosome classes in about the proportions of 20 percent of the former and 80 of the latter. The 24-chromosome microspores develop into pollen grains equivalent in every respect to those of normal plants; but the 23-chromosome microspores develop distinctively according to their specific chromosomal constitution; in some types they abort in early development, in others they give rise to defective pollen grains, exhibiting individual features as respects degree of development and character of contents; in very few they may follow approximately a normal course of development. The individual features of the pollen preparations, therefore, depend upon the course of development of the 23-chromosome class of microspores, and they are so distinctive that they provide a very satisfactory diagnostic feature.

The conclusion from studies of pollen preparations that the percentage of 23- and 24-chromosome microspores is approximately 80 and 20, respectively, instead of 50 of each type, is closely in agreement with studies of sporogenesis. Lagging and consequent elimination of the monosome in microsporogenesis has been described by OLMO (1935). The excluded laggard chromosomes form micronuclei which may be counted at the sporad stage. OLMO's estimates for the percentage of 23-chromosome microspores, based on such counts, were 76.8 for haplo-A, 75.6 for haplo-B, 76.7 for haplo-N, and 75.0 for haplo-R. Similar studies of megasporogenesis by FANSLER (1941), a more difficult task, led to estimates of percentage of 23-chromosome megaspores of the same order of magnitude—72.8 for haplo-F, 71.1 for haplo-H, and 74.3 for haplo-O; and in a recent paper GERSTEL (1943) has shown that each of two independent, unassociated chromosomes is included in only approximately 20 percent of the megaspores. It seems to be established, therefore, that elimination of the monosome in sporogenesis occurs to practically the same extent in each monosomic type, both in microsporogenesis and in megasporogenesis, setting up a primary ratio of about 80 to 20 for 23- and 24-chromosome spores, respectively, and that the differences among the monosomic types depend primarily upon the subsequent development of 23-chromosome spores.

Ovule abortion

The studies of ovule abortion are best conducted somewhat before the midpoint of the normal period from pollination to maturity of the seed, about ten to twelve days after pollination. Counts are readily made by removing a section of the outer wall of the capsule, exposing the developing ovules attached to the placental surface. Examination is conveniently made with a 20× binocular, using a small needle scoop to remove the crowded ovules as they are counted. Normal ovules at this stage are plump and have attained full size; aborted ones are mere scale-like, flattened, collapsed vestiges, still attached to the placental surface.

In contrast to the distinctiveness of pollen preparations, the studies of ovule development seem almost invariably to reveal simply a sharp distinction between ovules which appear to be developing normally and those which have aborted; but there are wide differences in percentages of aborted ovules.

The data in table 1 reveal that in a number of monosomics, twelve in fact, the percentages of abortion cluster around 80 percent, which, assuming that the 23-chromosome class of megaspores abort, is very closely in agreement with the general results of pollen studies. The remaining monosomic types exhibit ovule abortion values ranging from 15 to 56 percent.

The interpretation of the data on ovule abortion, however, is not simple. Not all of the 23-chromosome megaspores abort, even in those monosomic types which approach the 80 percent level of abortion, as is shown by their ovular transmission ratios of 5 to 20 or even more percent.

FANSLER (1942) has made some studies of ovule abortion in haplo-F, haplo-H, haplo-O, and a modified monosomic having a G-M translocation chromosome replacing a G and an M chromosome. Her results indicate that ovule abortion may arise from several causes, of which the most important were early degeneration of the endosperm, failure of megaspore development, and absence of pollination. The monosomics under study varied as to relative frequency of these phenomena. Thus the percentage of ovule abortion arising from early degeneration of the endosperm was found to be approximately 40 for haplo-H, 24 for haplo-F, and only 9 for haplo-O; but abortion from failure of pollination occurred to the extent of about 5 and 6 percent, respectively in haplo-H and haplo-O, as contrasted with 16 percent in haplo-F. There was some slight evidence of a modified Renner effect, as seen in occasional development of megaspores other than the chalazal one into embryo sacs. This phenomenon, however, occurred too infrequently, not more often than four percent in haplo-H, to be an important factor in the situation, and when it occurred it seemed to be a random matter as to which of the other three megaspores would develop. Unfortunately these studies did not include any representative of the class of monosomics exhibiting high ovule abortion. The mid-development counts reported in table 1 appear to be equivalent to abortion arising from endosperm degeneration, and the extent to which this phenomenon is selective is debatable, since haplo-H in which 45 percent of the ovules abort, still has a transmission rate approaching the maximum value, whereas haplo-S which has only 21 percent of ovule abortion, nevertheless shows a much more marked depression in the transmission rate. High-abortion monosomics, however, all have low transmission rates. The phenomenon of abortion as a consequence of endosperm degeneration is reminiscent of BRINK and COOPER'S (1941) somatoplastic sterility; but further investigations are needed to determine the extent to which it is selective. At any rate, it is clear that maximum values approach 80 percent as with pollen abortion and that the high-abortion rates are associated with low transmission of the monosomic condition.

Seed production

Seed-production values, entered in table 1, have been obtained from capsules from hand-pollinated flowers. The seeds from single capsules were weighed; the number of seeds per capsule was estimated by counting the number of seeds in a ten milligram sample. Naturally most of the monosomics produce fewer seeds per capsule than normal plants; but one, haplo-A, appears to produce more. On the whole, seed production is reasonably closely correlated with percentage of good ovules, especially in the group of plants with high ovule abortion, but the relations among the remaining types are rather erratic. The method of estimation of ovular abortion may be at fault, since the determinations were made in the broadest portion of the capsule near the base. There was some evidence of uneven distribution of ovular abortion in the capsules. Moreover the comparison makes the tacit assumption that potentially all types produce the same number of ovules, which certainly has not been proved.

Ovular transmission

The ovular transmission rates recorded in table 1 have all been obtained from crosses of monosomic ♀ × normal ♂. These crosses may at the same time have included differences in Mendelian characters, but crosses between distinct varieties, which usually exhibit hybrid vigor, have been excluded. The populations here included have all been grown from counted samples of seed germinated on filter paper, the seedlings then transferred to pots, and from them successively to flats and to the field. These precautions were taken in order to eliminate selective effects among seedlings, which previous experience with ordinary methods of propagation has shown to be very marked for some monosomic types. Germination percentages of the seeds of the various monosomic types are astonishingly high and do not differ significantly among themselves nor from normal seed. Despite all precautions, however, the percentage of transmission of most of the monosomic types exhibits a high range of variation, far too high to be ascribed simply to random sampling. Apart from this unresolved difficulty, each monosomic is characterized by a specific transmission rate. The range of difference extends from 5 to above 80 percent. The maximum values are attained in only a few types, notably A, E, and O, in which the monosomics apparently appear in the progenies in about the same proportion as that in which the 23-chromosome megaspores are produced. Particularly notable, however, is the fact that those monosomics which belong to the high ovular abortion class, still exhibit some transmission, thus making it possible to propagate them and to work with them.

Correlative behavior

In figure 2 we have attempted to show graphically the degree to which correlative behavior exists with respect to pollen abortion, ovule abortion, seed production, and ovular transmission. The data have been taken from table 1,

but some slight shifts in values have been made in order to prevent confusion of lines in the figure.

The main conclusion which may be drawn from this figure is that there is a group of 12 monosomics (G-I-J-L-N-P-Q-T-U-V-W-Z) which is set apart from

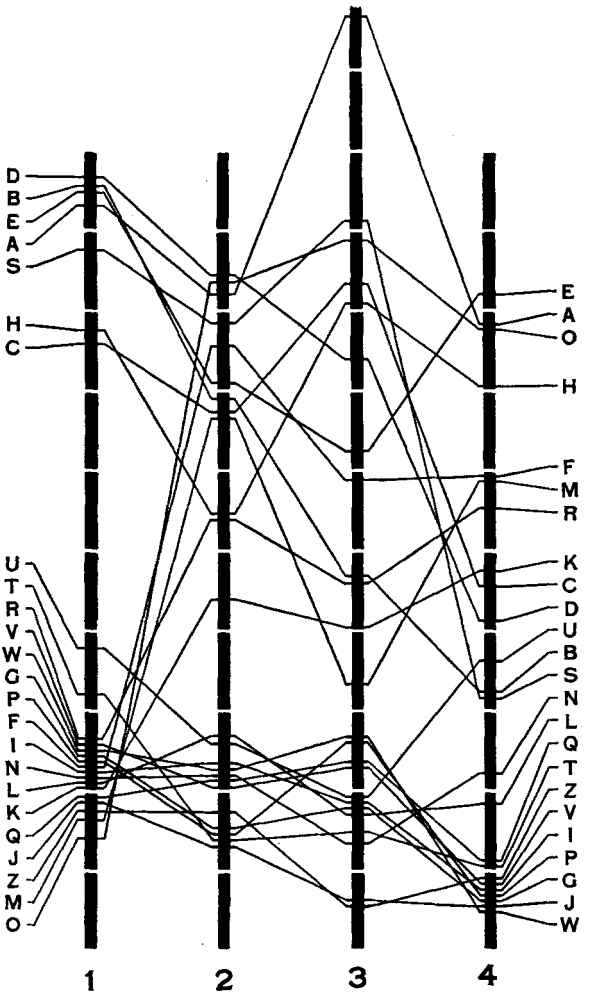

FIG. 2.—Relations among the monosomic types as to (1) percentage of good pollen, (2) percentage of good ovules, (3) seed production in percentage of normal set, and (4) ovular transmission rate from monosomic ♀ ×normal ♂. The vertical scales are interrupted at 10 percent intervals. Data from table 1.

the remaining types by low percentage of good pollen and ovules, low seed production, and low ovular transmission rates. There is a good deal of overlap as respects pollen conditions, since the halpo-types, F, K, M, O, and R are also listed as showing low percentages of good pollen; but in F, K, and O this classification is based upon a dimorphic condition of the pollen, rather than upon

complete abortion of the 23-chromosome grains. However, it is established that although pollen and ovular conditions are usually parallel, they are not necessarily so. Aside from that in pollen conditions, the two groups exhibit no further overlapping, except for the rather high recorded ovular transmission rate of haplo-U, which may depend in part on paucity of data for this type; more likely it depends upon inaccurate classification arising from confusion with variant types which appear in relatively high frequency in its progenies. Haplo-Z also produces a high percentage of variant types, but since its morphological features are more distinctive, there is less confusion in its classification.

In the other group of twelve types, there is no very pronounced correlative behavior within the group.

MONOSOMIC ANALYSIS

In monosomic analysis, associations in transmission of monosomic types with specific Mendelian factors replace the customary Mendelian ratios as evidence of the relations among factors and chromosomes. The method is not new, for it is exemplified in its essential features in sex-linked inheritance, where the heterogametic sex is monosomic for the sex chromosomes and hence hemizygous for factors located in it. With a complete set of monosomics, the well-known advantages of sex linkage in the study of genetic relations become applicable, at least in part, to all chromosomes of the set. The simplification accomplished by monosomic analysis is especially important in species with high chromosome numbers, since it permits accomplishment with them of something in the way of chromosomal analysis comparable to that which has been done with low-numbered species. More important still, it may make it possible to attack with some degree of precision the general problem of the genetic constitution of polyploid species.

Mendelian factors in Tabacum

Unfortunately a large number of simple Mendelian characters is not available in *Tabacum*, in which respect *Tabacum* appears to be a typical polyploid species. Those in our possession have mostly been transferred to our standard variety, Purpurea, by the method of recurrent backcrossing in order to have an identical residual background in our tests. This is not absolutely necessary for application of monosomic analysis, but it is at least advantageous, since complex segregation for quantitative features may interfere seriously with accurate classification of the monosomics.

The factors which have been tested thus far for chromosomal association are listed below. In assigning symbols to them, the convention has been followed of naming the factor from the character difference which it sets up from our standard variety, Purpurea; symbols with initial capitals represent dominant and those with lower-case initials represent recessive factors.

Br- broad: broad leaf base as contrasted with the sharply constricted leaf base of the standard type. The heterozygote is intermediate, but it is most convenient to include homozygous and heterozygous broad in one class.

co- coral: salmon-pink flower color associated with dwarf stature and small

grayish-green leaves. Coral is a deficiency character arising from substitution of a segment of the N chromosome for a segment of the non-satellited arm of the F chromosome.

cy- calycine: a teratological flower type with more or less extremely split corollas and enlarged, partially petaloid calyces.

*hf*1- *hf*2- hairy filament: a duplicate factor character from NOLLA's (1936) variety, Ceniza.

lf- light filaments: faintly colored filaments as contrasted with the dark filament color of Purpurea. Expression of the character is limited to plants having carmine flower color; pink and white flowering plants always have light filament color.

Ml- many leaved: a dominant character, transferred from *tomentosa*, marked by increase of five or six in leaf number. The heterozygote has the same leaf number as the homozygote.

mm- mammoth: the well-known growth type which exhibits marked photoperiodic response; indeterminate leaf number under long-day and determinate under short-day conditions, incompletely recessive to the normal growth type. Our mammoth race was established by transfer of the mammoth character from the commercial variety, Maryland Mammoth, to Purpurea.

pa- pale-sterile: a partially asynaptic type exhibiting an average of about eleven bivalents per microsporocyte; the rest of the chromosomes unassociated. Highly sterile, pollen almost completely aborted, seed production from pale-sterile ♀ × normal ♂ about five percent of the normal yield per capsule. Pale-sterile is completely recessive and must be carried along by selection of heterozygous plants.

Pd- petioloid: a petioled type with narrow, acutely pointed leaves and other associated features. Resembles petioled (*q.v.*) but is not so extreme either as to length of petiole or narrowing of the leaf. In combination with petioled, it produces a very extreme leaf type with a long petiole and long, very narrow, pointed blade.

pk- pink: light pink flower color as contrasted with carmine, to which it is almost completely recessive.

Pp- purple-plant: a dominant factor transferred from *tomentosa*. Stems, leaves, calyx and immature capsules exhibit a conspicuous, bronze-purple color. It requires exposure to sunlight for full development, but the associated purple style color seems to develop without such exposure.

Ps- purple-style: the style only has a more or less pronounced purple color. It should not be confused with the purple style color of purple-plant, which is inseparably associated with that character-complex.

Pt- petioled: this factor produces a complex of differences from normal, of which the narrow, petioled leaf type is most conspicuous. Associated differences include narrow, long-pointed calyx lobes, long-pointed corolla lobes, long, narrow, pointed capsules, semi-dwarf stature, and basal branching habit. The heterozygote is somewhat intermediate, but difficult to separate accurately from the homozygote.

rd- red: a dark modifier of pink such that *pk rd* has a dark red flower color almost equivalent to carmine. The heterozygote is pink, but more vividly colored than homozygous pink.

sg- stigmatoid: malformed anthers frequently tipped with a miniature style and stigma, together with associated plant features, particularly a moderate dwarfing of the plant and a reduction in flower size. The heterozygote is slightly intermediate as to flower size, but otherwise it is completely normal in expression. It is one of the X-ray types produced by GOODSPEED (1930).

wh- white: white as contrasted with colored flowers.

wh-P- pale: a flower color character; pale limb color, faintly colored tube and filaments. An allele of white transferred from *tomentosa.* It is recessive to full color but dominant to white.

ws- white-seedling: the seedlings are apparently totally devoid of chlorophyll and die soon after germination. It also is an X-ray type produced by GOODSPEED (1930).

yb1- yb2- duplicate factors for yellow burley, the familiar commercial character.

yg- yellowish-green: the partially chlorophyll-deficient character of NOLLA's (1934) variety, Consolation. A simple recessive to normal green.

The simplest genetic problem to which monosomic analysis may be applied is that of determining the location of factors in chromosomes. The type method is illustrated by results previously published in an earlier paper (CLAUSEN and GOODSPEED 1926b), as follows:

P_1 haplo-C carmine ♀ × diplo-C white ♂
F_1 haplo-C white + diplo-C carmine

The F_1 ratio is of no importance to the demonstration, it is the association of haplo-C with white which demonstrates that the factor for white is located in the C chromosome. A similar cross with each of the other 23 monosomic types gives an F_1 progeny which is carmine in both the monosomic and the normal classes, which just as positively demonstrates that the white factor is not located in any one of the other chromosomes.

The standard procedure for determining an association then is to cross the normal recessive with each of the 24 monosomic dominants, whereupon the results in general will be as follows:

P_1 haplo-X dominant × diplo-X recessive
F_1 haplo-X recessive + diplo-X dominant

if the factor is located in the chromosome, or if it is not:

F_1 haplo-X dominant + diplo-X dominant

The simplicity of the test and its advantages, as compared with the trisomic and other methods of determining such relations are so obvious as not to require elaboration. If dominant characters are to be tested, the dominant type is first crossed with each monosomic, in order to establish the necessary haplo-X dominant types, whereupon association is determined in the second generation

according to the pattern described above. In the case of complex factor situations, double recessives, homozygous lethals, complementary factors, etc., simple modifications of the procedure may easily be devised in order to establish associations, as is shown by some of the examples described below.

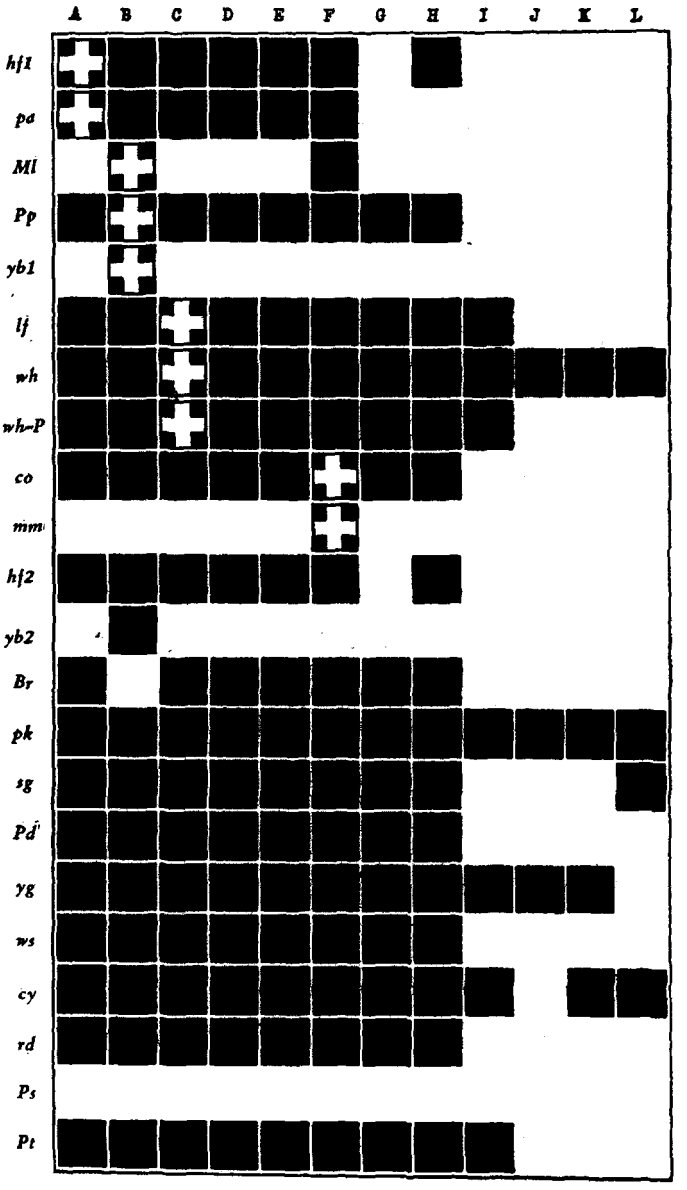

FIG. 3a.—Tests of factor associations with haplo-types of the *tomentosa* subgenome. The plus squares represent positive associations, the black squares negative results, and the blank spaces untested relations. Capital letter captions at the top represent chromosomes; italic letters at the left side, factor symbols as listed in the text.

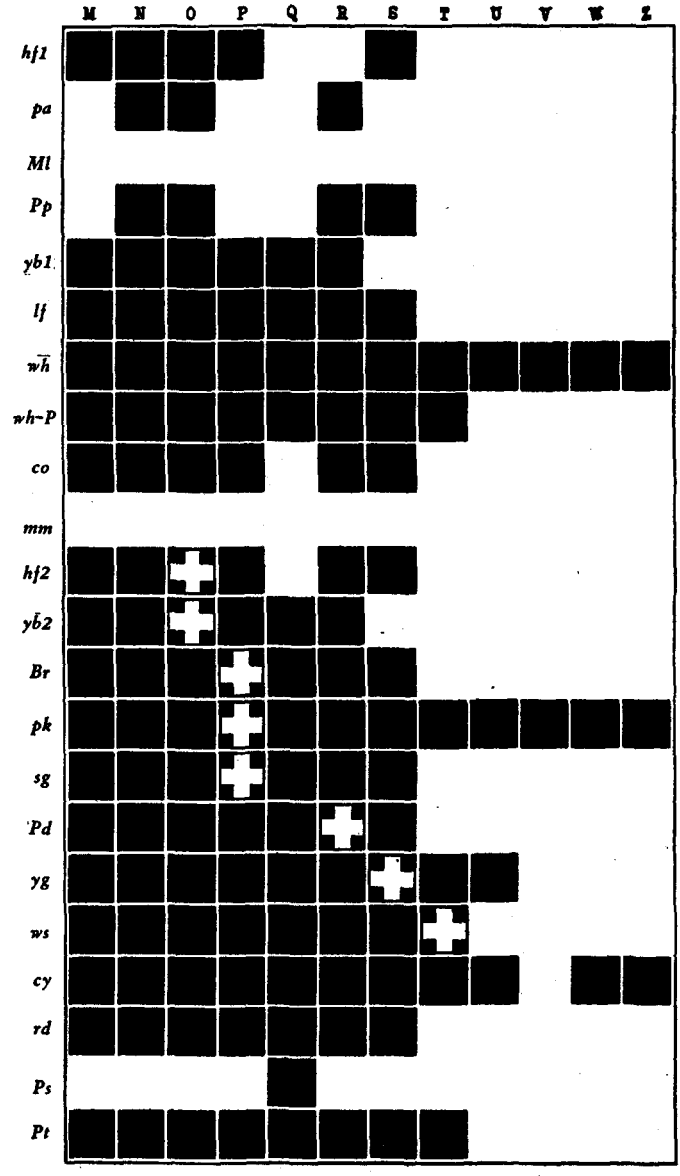

FIG. 3b.—Tests of factor associations with haplo-types of the *sylvestris* subgenome. Significance as in Figure 3a.

The results of 294 test crosses for association of factors with chromosomes are assembled in two diagrams (fig. 3a, 3b), in which a plus square indicates positive association; a black square, absence of association; and a blank space, an untested relation. In practice, tests for association are discontinued as soon as a positive association has been established.

The figure shows that the following factors have been located in specific chromosomes:

A	*hf*1	—hairy-filament
	pa	—pale-sterile
B	*Ml*	—many-leaved
	Pp	—purple-plant
	*yb*1	—yellow-burley
C	*lf*	—light-filament
	wh	—white flower color
	wh-P	—pale flower color
F	*co*	—coral flower color
	mm	—mammoth
O	*hf*2	—hairy-filament
	*yb*2	—yellow-burley
P	*Br*	—broad leaf-base
	pk	—pink flower color
	sg	—stigmatoid
R	*Pd*	—petioloid
S	*yg*	—yellowish-green
T	*ws*	—white-seedlings

SPECIFIC ASSOCIATIONS

The following accounts of results of tests for associations are, for the most part, limited to positive instances, since it seemed unnecessary to report details of negative results beyond the specific enumeration of the tests conducted, which are recorded in figures 3a and 3b. The details of positive associations are presented individually in order to illustrate the modifications of the type procedure which are necessary in order to meet specific genetic conditions.

Association of hairy-filament with haplo-A and haplo-O

Hairy-filament is a character exhibited by the variety Ceniza, described and distributed by NOLLA (1936). Acting under assumption that hairy-filament is a simple recessive character, we crossed our haplo-types with Ceniza and found in one case—namely, with haplo-A—that the haplo-A plants exhibited the character, but very weakly. The weak expression of the character might have depended upon the difference of genetic background, but when these haplo-A plants were selfed, they exhibited a ratio of approximately 3 normal: 1 hairy-filament, provided the hairy-filament class was restricted to those plants which showed a strong expression of the character. Sister diplo-A F_1 plants gave F_2 segregation approximating 15 normal: 1 hairy-filament. These results demonstrated that our variety Purpurea contained duplicate genes for the normal condition.

In view of these preliminary results, a further set of tests was conducted in order to determine the location of the second gene, since the preliminary results had shown one of them, *hf*1 to be located in the A chromosome. These tests were started by crossing the available haplo-types with hairy-filament, then selected F_1 haplo-types heterozygous for hairy-filament were selfed for production of F_2.

	smooth-filament	hairy-filament
A 41323	25	15
B 41325	47	1
C 41326	44	4

D	41327	40	2
E	41328	33	2
G	41329	36	0
H	41330	38	2
M	41333	39	5
N	41334	41	1
O	41335	30	15
P	41336	37	2
S	41339	37	1
Totals (excluding A and O)		394	20
Expectation 15:1		388	26

These results point to A and O as the chromosomes in which *hf*1 and *hf*2 are located, as is evidenced by the high proportion of hairy-filament segregants in the progenies of the F_1 haplo-types heterozygous for hairy-filament. Division of these progenies into normal and haplo-types has not been recorded in the table because it furnished no pertinent information as to location of the genes. Classification for the haplo-types was in some instances very uncertain, because of the considerable degree of segregation occurring in F_2 of crosses between Purpurea and Ceniza, the source of the hairy-filament character. Segregation for hairy-filament, however, was independent of the haplo-type division. Excluding A and O, the segregation is in satisfactory agreement with the 15:1 ratio for duplicate genes.

In the course of these tests it was noted that haplo-O heterozygous for hairy-filament exhibited a weak expression of the character, similar to that previously noted for haplo-A heterozygous for hairy-filament, and that apparently some other heterozygous conditions might likewise do so. The extent and the basis of these weak expressions have not as yet been entirely clarified, but in the homozygous condition the hairy-filament character is even more pronounced in our Purpurea derivatives than in Ceniza.

For a more complete exposition of the analytical features of the monosomic system of locating duplicate genes, the reader is referred to the account of yellow burley, following.

Association of pale-sterile with haplo-A

P_1 haplo-A normal ♀ × diplo-A pale sterile heterozygote ♂

		haplo-A pale-sterile	haplo-A normal	diplo-A normal
F_1	33128	17	5	6
	36170	7	21	12
	36253	12	18	8
	37238	16	21	5
	Totals	52	65	31

These results illustrate the method to be employed when it is not possible to use the homozygous type in the tests. Pale-sterile plants exhibit almost com-

plete pollen abortion, and their seed progeny is so variable, on account of the disturbed distribution of chromosomes, that it is impracticable to study transmission of the character except through employment of heterozygous plants.

Association of many-leaved and purple-plant with haplo-B

P_1		haplo-B many-leaved purple-plant ♀ ×
		diplo-B normal-leaved green-plant ♂
F_1	37240	22 haplo-B normal-leaved green-plant
		13 diplo-B many-leaved purple-plant

As noted in the list of factors, both of these factors have been transferred from *tomentosa* by the method of recurrent backcrossing. The transfer of the many-leaved type was an unexpected consequence of the result of transferring the more-obvious purple-plant character to our standard variety of *Tabacum*. After the transfer of the purple-plant factor had been accomplished it was noticed that the purple-plant type was taller and somewhat later than the standard type, and counts showed that it had five or six more leaves than the normal number. Linkage between the two factors is very close; to date backcrosses of heterozygous many-leaved purple-plant to normal-leaved green-plant have given only 3.6 percent of recombinations, as shown by the following results from seven such progenies: 125 many-leaved purple-plant, 144 normal-leaved green-plant, 8 many-leaved green-plant, 2 normal-leaved purple-plant.

Association of yellow-burley with haplo-B and haplo-O

The inheritance of yellow-burley of commercial burley varieties, as originally demonstrated by KAJANUS (1924) and more recently confirmed by HENIKA (1932), is dependent upon duplicate genes, so that F_2 ratios approximate 15 green: 1 yellow-burley. These results agree with our own determinations which employed the commercial variety Station Standup (MACRAE and HASLAM 1935) as the source of yellow-burley and our standard variety Purpurea as the green parent. The monosomic analysis demonstrates that one of the factors, *yb*1, is located in the B chromosome; the other, *yb*2, in the O chromosome.

The *yb*1 association with haplo-B is demonstrated by the following series of results. Haplo-B green ♀ × diplo-B yellow-burley ♂ gives two classes of F_1 offspring—haplo-B green and diplo-B green—both heterozygous for yellow-burley. These two classes of plants were then tested for yellow-burley segregation in progenies from selfing and from backcrosses to yellow-burley.

The results from F_1 diplo-B green plants selfed:

		F_1 diplo-B yellow-burley heterozygote × self	
		diplo-B green	diplo-B yellow-burley
F_2	41310	43	3
	41319	36	3
	Totals	79	6

And from the same plants backcrossed to yellow burley:

F_1 diplo-B yellow-burley heterozygote ♀ × yellow-burley ♂

		diplo-B green	diplo-B yellow-burley
Backcross	41312	34	10
	41321	30	10
	Totals	64	20

The results from selfing are in satisfactory agreement with 15:1 duplicate-gene segregation, and those from backcrosses are in agreement with the expected 3:1 ratio.

The tests of sister haplo-B plants for yellow-burley segregation on the other hand conform to the 3:1 ratio for selfing and the 1:1 ratio for backcrossing as shown by the following results:

F_1 haplo-B yellow-burley heterozygote × self

		diplo-B green	haplo-B green	diplo-B yellow-burley	haplo-B yellow-burley
F_2	41313	10	26	4	7
	41316	15	15	7	5
	Totals	25	41	11	12

F_1 haplo-B yellow-burley heterozygote ♀ × yellow-burley ♂

		diplo-B green	haplo-B green	diplo-B yellow-burley	haplo-B yellow burley
Backcross	41315	8	7	5	15
	41318	25	3	14	4
	Totals	33	10	19	19

The following reciprocal backcross offers further confirmation of 1:1 segregation:

diplo-B yellow-burley ♀ × haplo-B yellow-burley heterozygote ♂

		diplo-B green	diplo-B yellow-burley
Backcross	41314	18	19
	41317	18	22
	Totals	36	41

The significance of these results is apparent from the following factorial analysis:

$$\text{haplo-B green} \quad \frac{B - Yb_1}{B_0} \; \frac{O - Yb_2}{O - Yb_2}$$

$$\text{diplo-B yellow-burley} \quad \frac{B - yb_1}{B - yb_1} \; \frac{O - yb_2}{O - yb_2}$$

haplo-B green ♀ × diplo-B yellow-burley ♂:

$$F_1 \text{ haplo-B green} \quad \frac{B - yb_1}{B_0} \; \frac{O - yb_2}{O - Yb_2}$$

$$\text{diplo-B green} \quad \frac{B - yb_1}{B - Yb_1} \; \frac{O - yb_2}{O - Yb_2}$$

The F_1 haplo-B plants are heterozygous for *Yb2 yb2* only, whereas their diplo-B sister plants are heterozygous for both pairs of factors. In these formulae, the convention Bo is employed to represent the missing B chromosome in haplo-B.

For determining the chromosome which carries *yb2*, the assumption was made that inasmuch as *yb1* is located in one of the chromosomes of the *tomentosa* subgenome, the chances were good that *yb2* would be located in a member of the *sylvestris* subgenome. Consequently initial tests were confined to the haplo-types then available for that subgenome. The haplo-types were first crossed with yellow-burley, then selected F_1 haplo-types heterozygous for yellow-burley were backcrossed to yellow-burley. Evidently *yb2* is located in the O chromosome, as shown by the results recorded below:

		green	yellow burley	percentage yellow burley
M	42397	36	9	20
N	42398	28	8	22
O	42399	19	17	47
P	42400	33	9	21
Q	42401	32	12	27
R	42402	27	12	31
S	42403	27	4	13
T	42404	28	8	22
U	42405	37	8	18
Totals (exluding haplo-O)		248	70	22

These results illustrate the modifications in procedure necessary to employ monosomic analyses in the location of duplicate genes. However, they do not illustrate all the advantages which may be gained from it. Thus, if it be desired to establish types homozygous for each dominant gene singly, a direct method is available. Haplo-B green × diplo-B yellow-burley, followed by selfing

of the F_1 haplo-B plants and selection for green will establish the *yb*1 *yb*1 *Yb*2 *Yb*2 type, and *mutatis mutandis* the *Yb*1 *Yb*1 *yb*2 *yb*2 type may be produced from haplo-O green×diplo-O yellow-burley. The advantages are evident from consideration of the number of tests which must be made in order to insure establishment of these two monogenic green types by the traditional method. We hope to present later a more complete demonstration of monosomic analysis of yellow-burley inheritance.

Association of light-filament with haplo-C

P_1 haplo-C dark-filament ♀ ×diplo-C light-filament ♂
F_1 38252 29 haplo-C light filament
12 diplo-C dark-filament

The demonstration here conforms to the standard procedure. As noted in the description of the character, light-filament is only determinable in carmine plants. Most *Tabacum* varieties have pink flowers and as a consequence light filaments. To determine whether or not they are genetically light-filament, they may be crossed with haplo-C carmine, as above, and the presence of light or dark colored filaments in association with haplo-C will immediately reveal their genetic constitution with respect to this feature

Association of white and pale with haplo-C

Numerous demonstrations of association of white and pale are available of which the following are illustrative:

P_1 haplo-C carmine ♀ ×diplo-C white ♂
F_1 36092 21 haplo-C white+14 diplo-C carmine
37242 23 haplo-C white+20 diplo-C carmine

Association of pale with haplo-C is demonstrated by the following results:

P_1 haplo-C carmine ♀ ×diplo-C pale ♂
F_1 33136 25 haplo-C pale+7 diplo-C carmine
34082 19 haplo-C pale+7 diplo-C carmine

Like white, pale is recessive to carmine and segregates in F_2 in a simple ratio. When it is crossed with white, F_1 is pale, and F_2 gives a simple ratio of 3 pale: 1 white. Pale, therefore, is an allele of white. However, since pale is a transferred character derived from *tomentosa*, it possibly may not differ so simply from carmine and white as the formulation here adopted suggests, for the *tomentosa* segment in which it was transferred may differ in a number of factors from those in the corresponding *Tabacum* segment.

Association of coral with haplo-F

P_1 haplo-F carmine ♀ +diplo-F coral ♂

	haplo-F coral	diplo-F carmine
F_1 34164	7	37
35208	5	23
35209	11	11
Totals	23	71

Haplo-F coral plants are difficult to raise, especially under field conditions, so that, as in these cultures, the number of haplo-F plants is far below expectation. The evidence that coral is a deficiency character comes from both cytological and genetical studies and will be presented in detail in a subsequent paper. Briefly, diplo-F coral sometimes has multivalent associations involving as many as four chromosomes; and, although haplo-F almost always is 23II +1I, haplo-F coral exhibits 22II+1III in about one-fourth of the microsporocytes. Moreover, in haplo-N plants, containing a normal N chromosome, a normal F chromosome and a coral F chromosome, the chromosomes are associated in a chain trivalent in about two-thirds of the microsporocytes. From these and other considerations we may conclude that coral has a normal N chromosome, but that its F chromosome has become altered through exchange of a segment for a segment of the N chromosome.

Association of mammoth with haplo-F

P_1 a) haplo-F normal-growth ♀ × diplo-F mammoth ♂
b) haplo-F normal-growth ♀ × haplo-F mammoth ♂
F_1 a) 33334 22 haplo-F mammoth+8 diplo-F normal-growth
b) 35213 23 haplo-F mammoth+6 diplo-F normal-growth

These results are to be compared with those for coral listed above.

The relation between mammoth and haplo-F was one of the first to be established. It immediately suggested an efficient method of transferring a recessive character from one variety to another, which was applied to the transfer of the mammoth character from Maryland Mammoth to Purpurea, as follows:

P_1 haplo-F Purpurea ♀ × Maryland Mammoth ♂
F_1 haplo-F mammoth + diplo-F normal
B_1 haplo-F Purpurea ♀ × F_1 haplo-F mammoth ♂

followed by repetition of the backcross in successive generations until the Maryland Mammoth features were completely eliminated. Since each generation is backcrossed, progress is twice as rapid as it is when each generation of backcrossing is separated by a selfed generation for obtaining mammoth segregants, according to the customary program of recurrent backcrossing.

Association of broad with haplo-P

P_1 haplo-P constricted carmine ♀ × haplo-P broad pink ♂
F_1 38159 0 haplo-P broad pink
34 diplo-P broad carmine

The direct demonstration of the association of broad with haplo-P by means of a test cross of the following type,

P_1 haplo-P broad ♀ × diplo-P constricted ♂
F_1 haplo-P constricted + diplo-P broad

cannot very well be employed, because haplo-P constricted itself has a broad leaf base as a feature of its haplo-P condition. A further generation would

demonstrate the genetically constricted constitution of these haplo-P plants since their diplo-P offspring would all be constricted, but this test has not been made. The present test, however, is just as satisfactory. The haplo-P broad pink plant was obtained from the culture listed below, the offspring of a cross of haplo-P constricted carmine ♀ × diplo-P broad pink ♂. The fact that the male gametes of this plant all carried the broad factor demonstrates that it is located in the P chromosome. The total absence of the expected haplo-P broad pink plants does not affect the validity of the demonstration.

Association of pink with haplo-P

P_1 haplo-P constricted carmine ♀ × diplo-P broad pink ♂
F_1 37245 4 haplo-P broad pink
36 diplo-P broad carmine

Since broad and pink are both located in the P chromosome, they should exhibit linkage relations. The recombination value actually obtained in experiments conducted for this purpose was approximately 4.8 percent, as shown from the following summarized totals from fourteen progenies of broad pink/constricted carmine heterozygotes backcrossed to constricted pink: 289 constricted carmine, 272 broad pink, 15 broad carmine, 13 constricted pink.

It is also interesting to note that, just as a transfer from *tomentosa* was shown simultaneously to involve two linked factors, so one of broad from *sylvestris* to *Tabacum* included a factor for pink, although its presence was not revealed until the derivative lines were self-fertilized in order to obtain homozygous broad. These characters, however, already existed among our *Tabacum* stocks, whereas those from *tomentosa* represent new features in *Tabacum*.

Association of stigmatoid with haplo-P

P_1 a) haplo-P normal-anther ♀ × diplo-P stigmatoid ♂
b) haplo-P normal-anther ♀ × haplo-P stigmatoid ♂
F_1 a) 37256 2 haplo-P stigmatoid + 39 diplo-P normal-anther
b) 38160 2 haplo-P stigmatoid + 31 diplo-P normal-anther

Experiments to determine the linkage relations of stigmatoid with broad and pink are in progress, but results are not yet available.

Association of petioloid with haplo-R

P_1 haplo-R petioloid ♀ × diplo-R normal ♂
F_1 42371 28 haplo-R normal
18 diplo-R petioloid

The monosomic demonstration for a simple dominant requires two generations. The above tests started with a cross of each of the available monosomic types with diplo-X petioloid. From the F_1, haplo-X petioloid plants were selected and tested as above; the results revealed association of petioloid and haplo-X. They also might have been tested by selfing, which in view of the

hemizygous condition of R-chromosome factors would produce diplo-R plants homozygous for all of their R-chromosome factors.

Association of yellowish-green with haplo-S

P_1 haplo-S normal ♀ × diplo-S yellowish-green ♂
F_1 42372 20 haplo-S yellowish-green
24 diplo-S normal

The demonstration for yellowish-green leaf color follows the standard procedure precisely. The results confirm those of NOLLA (1934) as to the simple recessive basis of yellowish-green.

Association of white-seedling with haplo-T

P_1 haplo-T normal ♀ × diplo-T white-seedling heterozygote ♂
39097p6 × 146p4 1086 green seedlings
56 white seedlings

This test for white-seedling association essentially follows the pattern for pale-sterile described previously, inasmuch as green plants heterozygous for white-seedling must be used as the source of that character. In the above progeny, the white seedlings are haplo-T, and presumably an equal number of haplo-T green seedlings were present, consequently the 4.90 percent of white seedlings indicates a transmission rate of 9.8 percent for haplo-T, which is in satisfactory agreement with the value 11.0 recorded in table 1.

Haplo-T is an illustration of a monosomic isolated by design as the specific chromosome in which *ws* is located. The original tests revealed that *ws* was not located in any of the chromosomes for which monosomics were then available. The isolation of the desired monosomic was then accomplished by the following procedure. First, pale-sterile ♀ × green heterozygous for white-seedling ♂ gave seeds which were tested for white-seedling segregation. Presence of a few white seedlings indicated that the monosomic was viable. From a further cross of pale-sterile ♀ × calycine ♂, two populations were grown, together comprising 40 plants. These plants were crossed individually with a plant known to be heterozygous for white-seedling and at the same time also with normal Purpurea. Seedling tests of the former crosses disclosed two plants which produced some white seedlings. Populations were then grown from the crosses of these parent plants with normal. Both exhibited complex segregation, but in one of them a new haplo-type with distinctive features was recognized. Subsequent tests proved that it was monosomic for the chromosome in which *ws* was located, as was to be expected. Calycine was here employed for the sake of economy in the hopes of obtaining the type monosomic for the chromosome in which *cy* is located; but although one of the F_1 plants was calycine, hence presumably monosomic for this chromosome, it was of such complex constitution chromosomally that through some mischance we failed to identify the desired monosomic in its progeny.

Incomplete tests for association

The diagrams show that four genes—*cy*, calycine; *rd*, red; *Ps*, purple-style, and *Pt*, petioled—so far have given negative results for tests of association to which they have been subjected. Tests for none of them, however, have been completed. If it should prove that any one of them or of other factors now undergoing tests should fail to exhibit association with one of the twenty-four monosomic types, it would constitute *prima facie* evidence that our collection of monosomics did not contain all the primary types, but it would not show which particular one was at fault. The demonstration of a positive association, on the other hand, provides final proof of acceptability of a monosomic as a primary, unmodified type.

EXCEPTIONAL PROGENY

As we have noted previously, monosomics are a fertile source of variant chromosomal types. Such types are usually most effectively recognized in selfed progenies, but sometimes they appear also in analytical progenies of the kind reported in this series of investigations.

Occurrence of variegation types, presumably through spontaneous production of unstable ring chromosome fragments, has been recorded for the F chromosome, giving carmine-coral variegation (CLAUSEN 1930); for the B chromosome, giving purple-plant-green plant variegation; for the C chromosome, carmine-white (STINO 1940) and for the P chromosome, carmine-pink. Apart from the fact that they all exhibit the same general type of variegation—that is, dependent upon sporadic elimination of the dominant color factor during ontogeny—they are very diverse as respects transmissibility, stability and types of derivative products. Each one poses a cytogenetic problem of some complexity.

Other products which have been recognized and studied to some extent include fragment products of the monosome. In the case of the C chromosome two small fragments, one of which appears to be equivalent mainly to one arm of the chromosome and one to the other arm, appear rather frequently. A stock has been set up in which these two complementary fragments have been substituted for the normal C chromosome (STINO 1938). It consequently exhibits 25 II chromosomes in meiosis and breeds true for this condition. Morphologically it differs only in minor respects from the normal type.

Perhaps the most significant recurrent aberrancy which has been determined is an oscillating relation between monosomics and their trisomic counterparts. Crosses of monosomic ♀ × normal ♂ give a small percentage of trisomic counterparts; and similarly those of trisomic ♀ × normal ♂ in turn produce occasional monosomics. This oscillating disturbance in distribution of chromosomes provides a convenient way of producing the counterpart types and of matching them up. Haplo-C and triplo-C, haplo-F and triplo-F, haplo-N and triplo-N, and haplo-R and triplo-R have been observed to behave according to this pattern, and no doubt the others do also. It is not surprising to find that the morphological features of these trisomics differ from normal

in the opposite direction from that of their monosomic counterparts. Haplo-F and haplo-N are small-flowered types and triplo-F and triplo-N have large flowers; haplo-C which is large-flowered produces triplo-C which is distinctly small-flowered. This oscillating relation may also exist in basic species. EINSET (1943) has recently reported the presence of monosomics in trisomic progenies of maize, but their specific constitutions were not determined. The production of trisomics from monosomics, however, apparently presents some complications. About half of a number of independent instances of production of triplo-C have proved on test to have one C chromosome deficient for the color factor.

A number of other exceptional types of progeny have been secured; but since their constitution has not been definitely determined, further comments appear uncalled for at this time. As a matter of fact each of them presents an independent problem of considerable complexity, and many more of them appear than we have been able to analyze.

DISCUSSION

The foregoing account demonstrates the feasibility of establishing monosomic types in *Tabacum* and of employing them for locating factors in specific chromosomes. None of the tests has been conducted on a large scale; one small culture is first grown and repetitions are made only if the results are in doubt. Obviously one of the advantages of the system is its ability to yield pertinent evidence in small cultures. The conduct of the experiments, however, reveals some of the limitations to successful employment of the method. Most of the populations must be grown from analytical crosses; consequently species like wheat, in which procurement of sufficient seed by hybridization involves an inordinate amount of effort, would scarcely be suitable subjects for such investigations, even if, as appears almost certain, a complete collection of monosomic types could be established in them. The method is obviously limited to those species in which transmissible monosomics may be established, very likely limited therefore exclusively to polyploid species, although we should not be surprised to find that monosomics of basic species, so far recorded as non-transmissible, may on more extensive trial exhibit a low rate of transmission.

Even with *Tabacum*, which is so highly favorable a subject for hybridization, location of a factor is still a laborious task, requiring tests with 24 monosomic types. We have therefore sought to devise a method which would reduce the amount of labor necessary for this purpose. It is possible, now that we have become familiar with the features of the 24 monosomics, that the asynaptic type, pale-sterile, may be used for the purpose. A cross of pale-sterile with a simple recessive gives in all cases so far tried a few recessive-type plants in the progeny. These plants should, at least normally, be monosomic for the chromosome in which the recessive factor is located. Single plants are often difficult to identify, particularly, since in this case plants of the immediate progeny from pale-sterile are likely to have a somewhat complicated cytological constitution, but a further backcross of these recessive plants to normal should lead to isolation and identification of the monosomic in the next succeeding generation.

This modification of procedure would substitute two progenies for the 24 necessary in direct use of the monosomic types; but it could only be used for location of simple recessives, which unfortunately appear to be scarce in *Tabacum*.

Employment of the method immediately suggests other problems to which it may be applied. Thus if duplicate genes are truly duplicate and have the evolutionary significance which has been ascribed to them, determination of their locations should provide a method of establishing specific cross homologies between chromosomes of the *tomentosa* and *sylvestris* subgenomes of *Tabacum*. If we can trust the meager evidence thus far available, the hairy-filament analysis demonstrates a cross homology between chromosomes A and O; and that of yellow-burley between B and O. Thus the first results appear to indicate that cross homology between the two subgenomes is segmental rather than chromosomal.

This consideration of duplicate genes in turn raises the question as to why some of our available simple recessives, particularly mammoth, pale-sterile, white-seedling, and yellowish-green, are not also governed by duplicate genes. Certainly the two species which are presumed to have contributed to the ancestry of *Tabacum* must both have been normal as respects such characters, and as a consequence the original raw amphidiploid must have had duplicate genes for them. Now that we are in possession of raw amphidiploids equivalent to *Tabacum*, such as 4n-*sylvestris-tomentosiformis*, it should be possible to re-establish the duplicate gene constitution of *Tabacum* with respect to such characters and thus to enlarge the possibilities of exploiting this method of determining specific cross homologies.

A fascinating system of analysis based upon employment of monosomics unfortunately has been rather disappointing in practice, owing to the complex genetic basis of most of the character differences in *Tabacum*. Consider a variety and the problem of analyzing its total differences from the chosen standard variety. Crosses of the variety with each of the monosomic representatives of the standard variety should immediately reveal in F_1 any simple recessive characters which distinguish the two varieties and should at the same time locate the factors in the chromosomes. A second backcross to the monosomic types should disclose the existence of dominant factors in the various chromosomes. Since theoretically entire chromosomes may be transferred unaltered by repeated backcrossing to the standard variety, it would seem possible to handle effectively even relatively difficult problems of size inheritance and other types of quantitative differences by this method. We have also occasionally found the method useful in unmasking hidden characters, as for filament color, as previously noted.

Monosomic analysis may also be adapted to determination of chromosomal dislocations. In a recent paper MALLAH (1943) has shown that a number of *Tabacum* varieties exhibit translocations relative to Purpurea. If such varieties be crossed with the Purpurea monosomics, cytological studies of the F_1 monosomics should reveal which specific chromosomes have been altered by trans-

location. For if one of the chromosomes is subtracted from a ring of four, obviously it becomes reduced to a chain of three, which is the condition to be expected in those F_1 types which are monosomic for chromosomes which have participated in the translocation.

SUMMARY

The twenty-four monosomic types of *Tabacum* differ from normal and from one another in a specific ensemble of quantitative morphological features. Given a sufficiently uniform genetic background, they may be classified accurately on the basis of their morphology.

Monosomics may be established by design in a number of ways. Crosses of the asynaptic type, pale-sterile, with simple recessives permit direct production of types monosomic for the specific chromosomes which bear the recessive genes.

Thirty-five chromosome *sylvestris-Tabacum* counterparts also differ from normal *sylvestris-Tabacum* hybrids and from one another in a specific ensemble of morphological features, which may include qualitative as well as quantitative differences.

Study of the monosomes at MI disclose a wide range in size, as well as certain features of shape, which may aid in discrimination of types in some instances.

Variations in meiotic behavior include association of the monosome with a bivalent to form a trivalent, apparently largely limited to haplo-D and haplo-S; and promotion of non-conjunctional behavior in bivalents, the extent of which is apparently a specific feature of certain monosomic types.

On the basis of association of chromosomes in 35-chromosome *sylvestris-Tabacum* hybrids, monosomics may be classified into two groups, according as they are monosomic for chromosomes of the *tomentosa* or *sylvestris* subgenomes of *Tabacum*.

Pollen samples of monosomics exhibit specific differences, dependent upon the character and degree of development of the 23-chromosome microspores, which apparently uniformly constitute about 80 percent of the total number of microspores.

Ovular abortion rates of monosomics may reach a high level of about 80 percent as with pollen samples, and they are also characteristic for specific types.

Seed production rates for the monosomics are mostly characteristically depressed, especially in those which have high rates of ovular abortion.

Ovular transmission rates of the monosomics range from five to 80 percent. Monosomics which exhibit high ovular abortion rates show strongly depressed transmission values, but they still exhibit some transmission.

Studies of association in transmission between monosomics and Mendelian characters have led to location of 18 genes in nine chromosomes.

On the basis of location of genes for the duplicate factors concerned with hairy-filament and yellow-burley, a segmental cross homology is suggested between the A and O and the B and O chromosomes.

An oscillating relation exists as respects sporadic production of trisomic counterparts by monosomics, or monosomic counterparts by trisomics.

Attention is called to the possible value of monosomics in analysis of chromosomal dislocations as well as in determining the genetic basis of Mendelian characters.

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ADDENDUM

The reader's attention is called to the article by E. R. SEARS, Cytogenetic studies with polyploid species of wheat. II. Additional chromosomal aberrations in *Triticum vulgare*. Genetics **29**: 232–246, which appeared while this paper was in press.

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