

A CYTOGENETIC INVESTIGATION OF SEX EXPRESSION
IN SPINACIA

By
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Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
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DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology,
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AN ABSTRACT

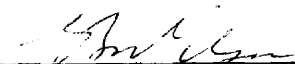
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Sex expression in the angiosperms, along with several cytogenetic explanations of sex inheritance in certain plants and animals, and the effect of environment are considered in the review of the general problem of sex expression and sex inheritance. The experimental work presented is divided into two parts: the cytological investigation and the genetic investigation.

The cytological investigation of Spinacia oleracea L. consisted of a study of meiosis in diploid male and intersex plants, and in tetraploid intersex plants. No evidence of a heteromorphic pair of chromosomes was detected. The chromosomal configurations of the diploid male were the same as those of the intersex plants; however, there appeared to be a difference between two intersex lines. One intersex line contained two nucleolar chromosome pairs, as did the male, and the other intersex line contained only one nucleolar chromosome pair. The tetraploid material examined indicated the presence of at least two trivalents at Metaphase I. Meiosis was otherwise regular up to tetrad formation, where it became abnormal

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ABSTRACT

by producing many-celled "tetrads" or polyads. The pollen produced from such polyads was irregular.

The genetic investigation consisted of four generations of breeding S. oleracea L., from which a scheme for sex inheritance was devised. Plant types and flower types were described in order to obtain a system of classification that would be suitable for genetic ratios. The genetic scheme devised consisted of the interaction of an X-and-Y chromosome mechanism and two compulsion linked genes, with a very low cross-over value, which were independent of the X and Y chromosomes. These two linked genes were equal in their sex-expressing capacity but were opposite in expressing sex type. A YY type of individual was considered to be viable.

The above-mentioned scheme was adapted to fit the conditions of sex inheritance found to exist in Spinacia; namely, the maintenance of a 1:1 sex ratio, the production of intersex plants, intersex plants which are pure breeding for the intersex condition, intersex plants which segregate into (a) intersexes and males, (b) intersexes and females, (c) intersexes and males and females.

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INTRODUCTION

The problem of sex inheritance in Spinacia oleracea L. has been attacked by using techniques of genetics and cytogenetics. Genetics is a relatively new science which has placed heredity on a mathematical basis involving the conception of the particulate nature of inheritance. Cytogenetics is the branch of science which is concerned with the mechanism by which these particles are carried from cell to cell, and from generation to generation. A clear concept of genetics has been given by Darlington (1951).

Genetics began as a study of the relations of parents and offspring in sexual reproduction. It continued by examining a deeper layer of events, the movements and activities of the determinants responsible for these relations. It then passed to consider unicellular and unimolecular organisms in which the distinction between determinant and phenotype almost lapsed and in which sexual reproduction entirely lapsed only perhaps to reappear again at a lower level. Genetic notions then transferred to the study of cell-lineages within organisms in which development took the place of heredity and differentiation took the place of variation. By these stages it will be found that genetics has gradually broken down the barriers between the departments of biology. It has done so by introducing those rigorous notions of constant particles constantly determining verifiable effects which Mendel so clearly set forth nearly a hundred years ago. Now indeed in 1950 we may claim to have rediscovered the elements which segregate, and recombine and, above all, determine.

He further adds, "Genetics removes the bandage from our eyes when we set out in pursuit of knowledge about living things."

The "bandage" in the case of sex expression in the angiosperms has been difficult to remove. Sex expression is a character which, in the cases of hermaphroditic and intersex individuals, is very difficult to place into specified classes. This, along with differing responses to environment, has made genetic analysis of the problem a difficult task. However, certain conclusions have been reached and are presented in this paper which may add evidence to the solution of the general problem of sex inheritance.

GENERAL REVIEW OF THE PROBLEM OF SEX EXPRESSION

Sex Expression in the Angiosperms

To what extent the dioecious and the monoecious complexes are present in the angiosperms has by no means a simple answer. Before this problem can be approached, it is essential, first, to define exactly what is meant by the dioecious and the monoecious complexes.

The following definitions, which are used throughout this paper, have been taken from a manuscript prepared by Gilly and Wilson (1952) which dealt with the problem of sex expression in the angiosperms.

1. Definitions applicable to the sexuality of flowers:
 - a. Perfect flower--A flower which is morphologically bisexual (i.e., containing both functional androecium and functional gynoecium).
 - b. Morphologically staminate flower--A flower containing only a functional androecium.
 - c. Functionally staminate flower--A flower containing a functional androecium and a rudimentary or otherwise nonfunctional gynoecium.

- d. Morphologically pistillate flower--A flower containing only a functional gynoecium.
- e. Functionally pistillate flower--A flower containing a functional gynoecium and rudimentary or otherwise nonfunctional androecium.

2. Definitions applicable to the sexuality of species:

- a. PERFECT--Species with all flowers on all individuals perfect.
- b. MONOECIOUS--Species with some flowers on all individuals morphologically staminate and other flowers on all individuals morphologically pistillate.
- c. POLYGAMO-MONOECIOUS--Species with all individuals bearing three kinds of flowers; perfect, morphologically staminate and morphologically pistillate.
- d. ANDRO-MONOECIOUS--Species with all individuals bearing both perfect and morphologically staminate flowers.
- e. GYNO-MONOECIOUS--Species with all individuals bearing both perfect and morphologically pistillate flowers.

- f. FUNCTIONAL MONOECIOUS--Species with some flowers on all individuals either morphologically or functionally staminate and other flowers on all individuals either morphologically or functionally pistillate; to fit into this category, either the staminate or the pistillate flowers on all individuals must be of the functional, rather than the morphological, type.
- g. DIOECIOUS--Species with all flowers on some individuals morphologically staminate and all flowers on the other individuals morphologically pistillate.
- h. POLYGAMO-DIOECIOUS--Species with some individuals bearing perfect flowers and either morphologically staminate or morphologically pistillate flowers and other individuals bearing flowers of the opposite morphological sexual type (accompanied or unaccompanied by perfect flowers).
- i. ANDRO-DIOECIOUS--Species with some individuals with only morphologically staminate flowers and other individuals with only perfect flowers.

- j. GYNO-DIOECIOUS--Species with some individuals with only morphologically pistillate flowers and other individuals with only perfect flowers.
- k. FUNCTIONAL DIOECIOUS--Species with all flowers on some individuals either morphologically or functionally staminate and all flowers on other individuals either morphologically or functionally pistillate; to fit into this category either the staminate or the pistillate flowers must be of the functional, rather than the morphological, type.

Some preliminary conclusions concerning the problem of the extent of the dioecious and monoecious complexes in angiosperms is presented by Gilly and Wilson (1952).

From the more than ten thousand genera which comprise over 150,000 species of the angiosperms, they indicated that at least 85,148 species come from families which contain some sort of dioeciousness, and 35,571 species are from the families which contain some truly dioecious species. The sixteen strictly dioecious families comprise 530 species.

The families which contain monoeciousness of one sort or another comprise 102,797 species, and those families which contain some truly monoecious species comprise 22,279 species. The twelve strictly monoecious families comprise 185 species.

They concluded from their investigations that at least 2,500 to 3,000 species are dioecious, and 7,000 to 7,500 species are monoecious, in the strict sense of the definition included in this paper.

The perfect condition (35,479 species) dominates the angiosperms, but the dioecious complex is by no means a small minority.

Another question which arises concerns the place of the dioecious and monoecious complexes in the evolutionary relationships between the types of sex expression. Five proposed systems of the evolution of these complexes are given.

One, based upon the systems of Bentham and Hooker (1862-1883), Bessey (1919), and Hutchinson (1926, 1934), shows a trend from the perfect condition to the monoecious or the dioecious condition; i.e., P to M or D.

Two interpretations are based upon the system of Engler and Prantl. One interpretation shows a trend from the dioecious

condition to the perfect condition, either through or around the monoecious condition; i.e., D to P direct, or through M. The other interpretation is a trend from both the dioecious and monoecious to the perfect condition; i.e., D and M to P.

To complete the series, Gilly and Wilson have added two more possible systems, one of which shows a trend from the monoecious condition to either the perfect or the dioecious condition; i.e., M to P or D; and another showing a trend from the perfect and dioecious condition to the monoecious condition; i.e., P and D to M. They conclude that the present data are simply not adequate for any decision at this time. However, they agree that differences of sex expression in the angiosperms are correlated with the genetic constitution of the organisms, and the problem of sexuality in the angiosperms is, therefore, to a high degree a genetic problem.

Further evidence for the genetic control of sex is shown by studies on the inheritance of flower types in the Cucurbitaceae and in maize.

From a series of crosses and back crosses in Cucumis and Citrullus, Rosa (1928) established that the differentiation of gynoecious and hermaphroditic flowers depended upon a single

genetic factor, hermaphroditism being recessive and andromonoeciousness being dominant.

Poole and Grimball (1939) concluded that in Cucumis melo the hermaphroditic plants differed from monoecious plants by two recessive genes. A plant heterozygous for one pair of genes is andromonoecious, and one heterozygous for the other pair is gynomonoecious.

Jones (1934) developed dioecious maize and propagated it through four generations. Two classes of sex-determining gametes were produced by the male plants; the female plants were monogametic. Double recessives of tassel seed-2 (ts_2) and silkless (sk) were found to be indistinguishable from tassel seed-2 by itself. Apparently, this tassel-seed gene had the ability to nullify the action of the silkless gene. Plants having the composition ($sk-sk-ts_2-ts_2$) produced seeds both in the lateral as well as the terminal flowers, and were female in function. When such plants were crossed with silkless individuals heterozygous for tassel seed-2 ($sk\ sk\ Ts_2ts_2$) which were male in function, the result was a progeny made up of the same two classes as the parents. Sex expression in this race of dioecious maize is therefore controlled by a single gene (Ts_2).

Emerson (1924) agreed that sex is probably an expression of the interaction of several, perhaps many, factors located in different chromosomes. He assumed that characters in general, including sex expression, develop through the cooperative influences of genetic factors and factors of the internal, as well as the external, environment. He added that in prevailing dioecious forms of the flowering plants, factors for maleness and for femaleness presumably are present in both male and female individuals, but here the balance is more strongly in favor of one or the other condition. The approximate numerical equality of individuals of the two sexes in these forms at once suggests a chromosome mechanism similar to that known to exist in numerous animal groups. The occasional appearance of sex intergrades approaching the condition of typical hermaphrodites may well be due to the influence of several heterozygous sex factors of relatively minor influence.

In his review of sex expression in angiosperms, Allen (1940) cited the genetic basis for sex expression as formulated by Correns in 1928, which assumed that all angiosperms possess potencies for femaleness, carried by a gene or gene-complex (G); and for maleness, carried by a gene or gene-complex (A).

Another gene or gene-complex (Z) influences for each species the time and order of appearance of male or female organs. In addition, a dioecious species has genes ("realizators") tending respectively toward femaleness (gamma) and toward maleness (alpha). This very broad hypothesis has been the basis of a number of explanations of sex inheritance in the higher plants.

Environment as a Factor

The environmental influences upon the sex expression is a problem which does not have a concrete answer. It is impossible to show that environment has no effect or that it has a 20-percent effect or is a primary controlling factor. Certainly, environment has an effect, for without environmental change, no life would exist. For example, spinach seed, if kept in a dry container, would eventually die, or if they were kept in a warm, moist, dark condition constantly, they would initiate growth, but soon die. In order to maintain growth and development through its complete reproduction cycle, a spinach seed must go through a series of ever-changing environments. It

is these ever-changing environments which permit the genetic complex to express the characters of the organism.

The degree of influence which environment has over the expression of characters is another question. Schaffner (1927) took the extreme view, and concluded that sex was purely physiological, and in no way dependent upon segregation and combination of the units of chromosomes. He placed emphasis upon external factors such as light, temperature, and soil, and believed that either staminate or pistillate plants carried within themselves the potentialities of expression of the opposite sex. This latter belief of Schaffner is usually accepted, but in the case of certain dioecious plants, the conditions necessary to realize the potentialities of changing sex would be so severe that it would result in the death of the organism. From observations in spinach, it appears that certain male types are so completely "overbalanced" on the androecious side that environmental changes, no matter how severe, would not change the sex of the plant. The same is true of the strong female plants. This compares with the conditions existing in most animals.

As the strengths of the androecious and gynoecious conditions tend to balance each other, environment becomes an

important factor. Evidence of this is shown in the effects of the winter (greenhouse) generations compared to spring (field) generations. Three lines of intersexes differed markedly in their sex expression, while dioecious strains maintained the 1:1 ratio under similar conditions. Sugimoto (1947) noted differences correlated with environments of spring and autumn plantings. The autumn plantings were influenced to a lesser degree than were his spring plantings.

The andromonoecious plants of Citrullus vulgaris used by Rosa (1928) were more sensitive to environmental conditions than were the andromonoecious plants of Cucumis sativa, as the stamens of hermaphroditic flowers were not always equally developed. In other varieties of monoecious cucurbitaceae, Whitaker (1931) reported that the ratio of male flowers to female flowers varies with season, and also with variety.

However, when the hermaphroditic or monoecious condition becomes genetically stabilized, environment once more becomes a minor factor. It is mainly when a species is in the process of changing genetically, due to controlled breeding, that environment exerts its greatest influence on the expression of characters.

Cytogenetic Interpretations of Sex Expression

In animals and dioecious plants there must be a system whereby one sex will not predominate and possibly eliminate the species. Disjunction between heteromorphic sex chromosomes is one system whereby the two sexes will be maintained in approximately equal numbers. Where no heteromorphic chromosomes are visible, and all chromosome pairs appear to be homologous, the presence of a crossover suppressor for a segment of a pair of chromosomes will give the same action as if the chromosomes were nonhomologous; that is, a 1:1 ratio for the particular segments is maintained. If these segments are comprised of sex-determining genes, then there is in effect an X-Y mechanism without heteromorphic pairs of chromosomes.

McClung (1902) studied the males of several orthopterans and suggested that the unpaired or "accessory" chromosome was the male sex determiner. Later, in the female, he found this chromosome was present twice--XX female; XO male.

Wilson (1905) studied the insect Lygaeus, and found in the female two X chromosomes, but in the male there was an unequal pair, one being an X chromosome and the other being

smaller. This was called the Y chromosome--X female; XY male.

In birds and lepidoptera it is the male which is homogametic, and the symbols Z and W are used--ZW female; ZZ male--or the W may be absent, the female being ZO.

Bridges (1921, 1922, 1925, 1932), working with *Drosophila*, found a triploid female which, when crossed with a diploid male, produced intersexes. His concept of genic balance or sex index was formulated to show the relation of X chromosomes to the autosomes. More exactly, the net male tendency of a set of autosomes is less than the net female tendency of an X chromosome. He suggested that the X chromosome (female tendency) be represented by 100 and the autosomes (male tendency) be represented by a lesser amount, approximately 80. From this he calculated a sex index in which 1.25 or larger was female, 0.63 or lower was male, and between 0.63 and 1.25 was the range of intersexes (Table I).

In the dioecious plant Rumex acetosa, fertile hermaphroditic types are described by Ono (1935) in a series paralleling the intersexes of *Drosophila*. Since polyploidy in Rumex has been pushed higher, to the heptaploid, there is an even greater

TABLE I
SEX INDICES IN DROSOPHILA
(after Bridges)

Sex	Formula	X 100	A 80	Sex Index
Super female	2N--3X2A	300	160	1.88
Female	4N--4X4A	400	320	1.25
Female	3N--3X3A	300	240	1.25
Female	2N--2X2A	200	160	1.25
Female	1N--1X1A	100	80	1.25
Intersex	4N--3X4A	300	320	0.94
Intersex	3N--2X3A	200	240	0.83
Male	2N--1X2A	100	160	0.63
Male	4N--2X4A	200	320	0.63
Super male	3N--1X3A	100	240	0.42

variety of types. However, all types with a sex index of 0.63 or lower were male, those with a sex index of over 0.63 and under 1.25 were intersexes, and all types with a sex index of 1.25 were females, except for one case. This case was a triploid which contained three X chromosomes and one extra autosome ($3X3A + a$) and was an intersex. However, another individual of the same formula ($3X3A + a$) was a female. This suggests that the autosomes differ amongst themselves in the potency of the male genes, and perhaps the major part of the male effect is due to a particular two of the six autosomes.

In two separate races of Lebistes, Winge (1922-1934) found a marked inherited tendency toward the production of females with male-like gonopodia. These "masculinized" females were further peculiar in that the X-linked patterns, for which they were heterozygous, showed up faintly. The normal formula for the two sexes is XX female, and XY male. By crossing together these inherited male tendencies, the two sets augmented each other and resulted in a small proportion of XX individuals that were genetically, phenotypically, and functionally males. They showed characteristics carried by the X of the father and also by the X of the mother. When these XX males

were crossed to standard females, all of their offspring were females, XX in constitution and supposedly heterozygous for about half of the masculinizing genes present in the autosomes of their father. After three generations of backcrossing these XX males to their daughters, a completely male individual appeared among the offspring. When this XX male was crossed to sisters, about half of the offspring were males.

Thus, a new race was established, homozygous for the X and free from the Y. In this race the X chromosome has stopped being the sex-differential chromosome and the X-borne characters are now inherited equally from both sexes, and are therefore "autosomal" in type. Sex differentiation has been transferred to a chromosome that was formerly an autosome, through the accumulation of male-tendency genes contributed by two separate races which had been selected for their masculinizing effects, until their influence was strong enough to give decisive differentiation. The new race is female heterogametic, while the old race is male heterogametic.

As the result of these findings, Winge concludes that numerous female-tendency and male-tendency genes exist in both autosomes and sex chromosomes alike, and the sex is the

outcome of the specific balance between these genes, among which there is no valid distinction as to 'primary' sex genes versus 'modifiers.'

Sex determination in hymenoptera and in a few other forms has long been formulated as N male and 2N female. If 1N gives a male, then twice the same N should also give a male. Perhaps the error lies in the assumption that the same two sets of chromosomes are present in the female. Inasmuch as the female arises from fertilization, her constitution may always be N/N' while the male is either N or N'.

Occasionally, biparental males are observed in Habrobracon; Whiting (1933) developed an hypothesis on the assumption that the normal female is a heterozygote between two differential pairs of factors, or multiple complexes of factors, which may be designated X^a and X^b , respectively. Normal males are of two genotypes, one carrying the X^a complex and the other the X^b complex, or chromosome. The biparental males would thus be $X^a X^a AA$ or $X^b X^b AA$, with the same genic balance as the normal haploid males, that is $X^a A$ or $X^b A$. The females would have a distinctive balance $X^a A/X^b A$, or the female is N^a/N^b and the males N^a or N^a/N^a , N^b or N^b/N^b .

Then the postulate that 1N results in male and 2N in female is not the case, but 1N and 2N are both male while the female is NN'.

In the case of Habrobracon, no direct effect on intersex is shown, but due to its interesting and complex genetic make-up, the above-mentioned scheme is important to the genetic balance of sex-determination theory.

Goldschmidt (1915-1934) has worked out one interpretation of sex inheritance in the moth, Lymantria dispar. He has found two races, one of which is weak (European) and the other a strong (Japanese) race for sex. He has formulated the interpretation that M (male) gene is located on the Z chromosome and the F (female) gene is in the cytoplasm of the female, thus F:M = female; F:MM = male; or ZW = female; ZZ = male.

However, a strong F with weak MM results in an intersex, and a weak F with a strong M also results in an intersex.

Winge (1937) sharply criticized Goldschmidt's formulation and offered an alternative explanation on the following basis:

1. The Z chromosome contains a net masculine tendency, strong (M50) in the Japanese race and weak (M10) in the European race.

2. The W chromosome has a net feminine tendency, very strong (F70) in the Japanese race and weak (F24) in the European race.

3. All autosomes carry sex genes, some of which pull in a masculine direction, some in a feminine; in the Japanese race the feminine autosomal factors are markedly preponderant (F20), but only slightly so in the European race (F4).

Warmke (1946), working with polyploidy in Melandrium dioicum, found that sex was determined by the X/Y ratio, and that the autosomes contributed little or no effect. Melandrium has distinct heteromorphic sex chromosomes that can be counted, and the ratios determined in polyploids. It was an XXXX/Y plant which was intersex, indicating that the Y was strong in male-determining genes, while the X was weak in female-determining genes, although XX/Y and XXX/Y show occasional intersex flowers.

Doris Love (1942) mentioned the possibility that the cause of one type of intersex in Melandrium was due to a translocation between the X and the Y chromosome. From measurements of the X and Y chromosomes of the intersex she suggested that it was originally an XX composition, but that a

section of the Y chromosome was translocated on to the short arm of the X.

These are but a few of the reports where sex determination has been studied, but they show definite patterns which should be considered in formulating a genetic basis for sex expression. However, the complexities of sex expression may be of such a nature that many unrelated systems may exist in the many and varied patterns of sex.

CYTOLOGICAL INVESTIGATIONS OF SEX EXPRESSION IN SPINACIA

Diploids

Review of literature. Winge (1924) examined the chromosomes in Spinacea oleracea L. and found no heteromorphic pairs. Nevertheless, he concluded that one of the six pairs of chromosomes must be sex-determining, where the X and Y are alike, and of the same appearance as the autosomes, and can consequently show crossing over.

Other investigators of the cytology of S. oleracea who failed to find heteromorphic chromosome pairs include Tuschnjakowa (1929), Sinoto (1929), Haga (1934), Lorz (1937), and Araratjan (1939).

Haga examined the macrospore meiosis, as well as microspore meiosis, and found no differences between the two sexes. However, Araratjan did show the presence of a heteromorphic pair in S. tetandra Stev. which had six pairs that resembled the six pairs in S. oleracea, except for the heteromorphic pair. Dolcher (1949) karyotyped S. turkestanica M. M. Ilijn, but he made no mention of the presence of heteromorphic chromosome

pairs. He found six pairs, but could distinguish only the satellited and the longest chromosomes with certainty.

There has been much disagreement as to the relative sizes of the chromosomes in Spinacia oleracea: Sinoto (1929) found one large, two medium, three small; Tuschnjakowa (1929) found three large and three small; Haga (1934) showed some differences in size, but did not place them in any particular order. Lorz (1937) described them as two large, two medium, and two small; Araratjan (1939) did not place the chromosomes of S. tetrandra into size classifications, but his drawings indicated that there was one large (heteromorphic), two medium, and three small pairs, which is roughly as Sinoto grouped them for S. oleracea. He also suggested the possibility of karyoraces occurring within Spinacia.

In conjunction with the genetical study of sex inheritance in spinach, a cytological study was also initiated to determine any differential chromosomal arrangement specific for any given sex type.

Materials and methods. The source of material for this study came from two unrelated intersex plants and pure male plants. One line was a pure-breeding intersex line developed

by Mr. W. Ferguson, of the Canadian Department of Agriculture. This line was designated C-1. The other intersex line originated from an open pollinated intersex plant followed by two generations of selfed intersexes. Intersex plants selected from the progeny of the second generation of selfed intersexes all bred true for the intersex condition. This line was designated L-15. The pure male plants were selected from a planting of the commercial variety, Long Standing Bloomsdale, which was the variety from which both intersex lines originated. The pure male plant was designated A-10.

Anthers were taken from the flowers of pure male plants, and from intersex plants. Whole flower clusters were fixed in a 3:1 absolute alcohol-glacial acetic acid solution for from 12 to 24 hours at room temperature. From these "fixed" flower clusters the anthers were easily dissected out under a dissecting microscope. Preparations were made using the iron acetocarmine technique and made permanent with diaphane.

For the somatic divisions, root tips were taken from freshly germinated seeds and placed in a 0.01 percent aqueous solution of colchicine for 2 hours before being fixed in the same

manner as the anthers. Aceto-carminc smears were then made, and the slides were made permanent with diaphane.

Meiotic observations. The gametic chromosome number of six ($2n = 12$) was found, and is in agreement with all other reports on the chromosome count of Spinacia oleracea. It is difficult to distinguish meiotic chromosomes on a strictly size basis, but diakinesis configurations are quite characteristic (Figure A). There appears to be one large pair of chromosomes with subterminal kinetochores whose arms form a configuration that varies from a right angle to a straight line. Two smaller chromosomes with submedian kinetochores, one of which forms an (X) with two short arms, and the other one forms a (Y) or an open 270-degree arc. One medium chromosome often forms a ring, and two smaller chromosomes are associated with the nucleolus in lines A-10 and L-15. Where only one chromosome is associated with the nucleolus, the other one forms a small ring.

Meiosis is regular and the nucleolus is conspicuous at pachytene, and remains about the same size until late diakinesis, after which it disappears.

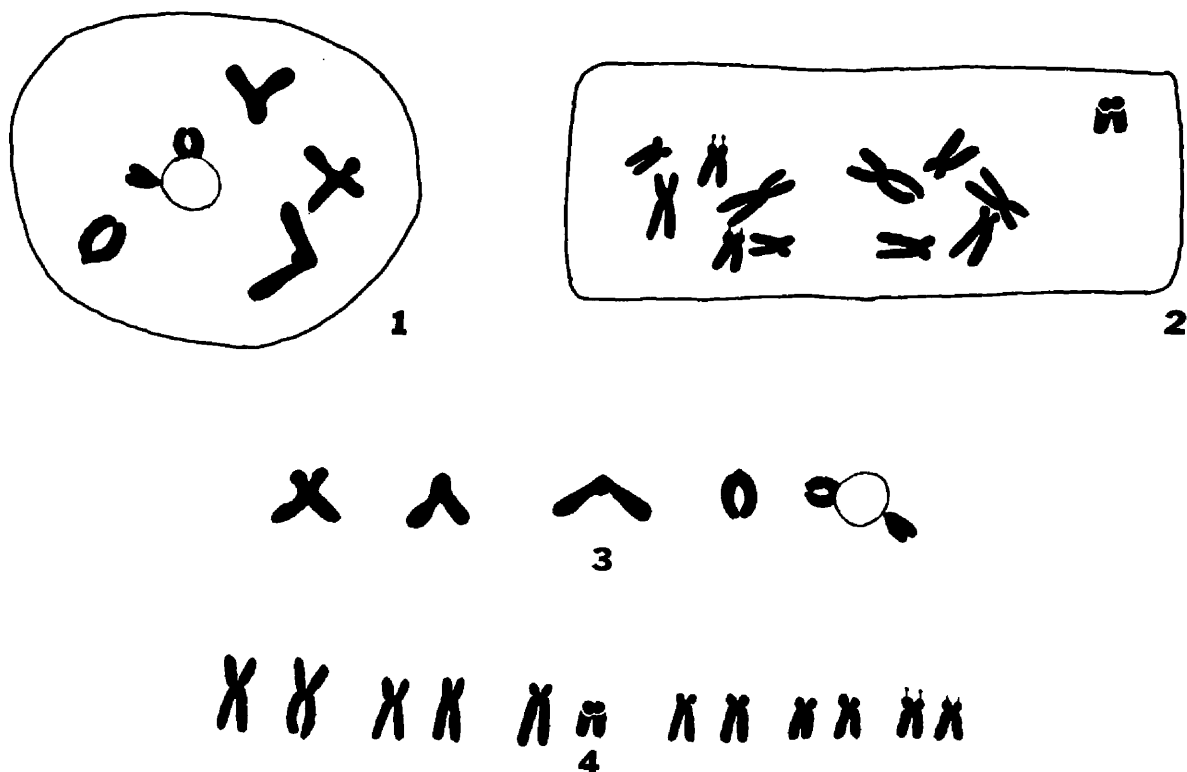


FIGURE A

KARYOTYPE OF SPINACIA OLERACEA L.

1. Diakinesis, Pollen mother cell (A10).
2. C--mitosis, Root tip cell (A10).
3. Karyotype of meiotic chromosomes.
4. Karyotype of somatic chromosomes.

The orderly arrangement of meiotic chromosomes and somatic chromosomes shows the relative size differences and the location of the kinetochores between the chromosomes of the same cell and between meiotic and somatic chromosomes.

At diakinesis, the two small pairs of chromosomes are associated with the nucleolus in plants of lines L-15 and A-10 (Plate I, No. 3), but only one of them appears to be so associated in C-1 line. Only one of the two small pairs of somatic chromosomes clearly shows trabants. Hardh (1939) found that in nine varieties of spinach, six varieties had one pair of satellited chromosomes, while three varieties had two such pairs. It is possible that both nucleolar chromosomes found in the L-15 intersex and in the pure males are satellited chromosomes, but the trabants are so small on one pair that it is difficult to show them.

At Metaphase I it is difficult to distinguish the different chromosomes. Two with the submedian kinetochores, and two small ones which are first to separate, can be distinguished (Plate III, No. 4). At no stage is there any evidence of the existence of a heteromorphic pair in any line examined. It may be pointed out, however, that failure to find such a pair is no evidence that such a pair does not exist. The difference may be too small to be resolved, or may consist only of a differential pairing segment.

There is no true interkinesis, the chromosomes going more or less directly from Telophase I into Metaphase II. At Metaphase II the chromosomes often arranged themselves in a circle of five, with the smallest one in the center, and this small one is often the first one to move out from the metaphase plate. Telophase II and tetrad formation proceed in a regular manner.

Except for the difference in the nucleolar chromosomes, meiosis was the same in the pure male and both intersex lines.

Tetraploids

Review of literature. Tetraploid spinach has been obtained by Tandon (1951), Warmke and Blakeslee (1939), and other investigators, but a cytological study has not been reported.

Tandon (1951) counted the Anaphase I chromosomes, but made no mention of further meiotic observations. He did note that the pollen sterility was 15 to 40 percent, but made no mention of abnormal pollen.

Materials and methods. The tetraploid plants used in the cytological study were induced from diploid seed of a strain of pure-breeding intersex plants by the use of colchicine. The seed was soaked in water for 24 hours, after which time the

radicles had emerged to about an eighth of an inch, and were then soaked in a 0.2-percent aqueous solution of colchicine for an additional 24 hours. They were then planted in soil in clay pots. Out of one hundred seeds thus treated, there were six which showed some effect of the treatment, but only two of these survived, both of which were intersex plants.

The growth was very slow, and after six true leaves were formed, the plants were placed in a 16-hour photoperiod which stimulated flower-stalk development, so the full vegetative stage of the plants was never attained. However, the largest of the leaves which were developed were heavily savoyed and very thick. As the flower stalk formed, the plants had a coarse appearance. The stem was thick and the leaf petioles tended to split where they were attached to the stem. Both plants were intersexes and set a "normal" number of seeds. The plants were heavily branched, which was characteristic of the strain from which the seed was taken. One plant had its main flower stalk fasciated to a thickness of 2 inches, and then the fasciation separated into many growing points as the plant approached maturity. The seeds were slightly larger than the diploid seed, and the seed coat was conspicuously wrinkled.

The plants continued to develop about two-thirds as rapidly as a diploid plant which was grown under the same conditions.

The material for the study of meiosis was prepared in the same manner as it was for the diploids. The pollen mother cells were easily broken, so more care had to be taken in the preparation of smears than was necessary with the diploids.

Meiotic observations. The chromosome count was $2n = 24$, as shown at Metaphase I and Metaphase II (Plate III, No. 2, 3). At Metaphase I there are at least two trivalents (Plate III, No. 7). However, no attempt was made at this time to analyze the chromosomes as to the number of univalents, bivalents, trivalents, or quadrivalents. There were, however, a considerable number of lagging chromosomes and bridges present. Meiosis, nevertheless, was regular, for the most part, up to the formation of tetrads. Soon after the Telophase II chromosomes began to lose their identity, cytokinesis was initiated for the formation of pollen grains. The pattern of cytokinesis was usually found to be irregular, which results in a number of microcytes in the "tetrad" (Plate II, No. 8). Wilson (1946) described the formation of microcytes in addition to the normal four cells in the tetrads of triploid and tetraploid banana, and

attributed them to independent division of "omitted" or "lagging" chromosomes. While there are certainly chromosomes which are "omitted" or "lagging" in this material, there are in many cases more cells per "tetrad" than one would expect from the average number of second-anaphase laggards. Also, there are as many odd-celled "tetrads" as there are even-celled ones (Table II).

The pollen developed from these cells is just as variable in size as the cells in the tetrad, the smallest microcyte being able to develop thickened walls characteristic of normal pollen grains (Plate II, No. 9).

The "tetrads" counted were taken at random, from one transverse sweep of the slide containing several anther smears, using the high dry lens. All cells which could accurately be counted were counted as they appeared in the field.

In Tetraploid C there were eight "tetrads" which contained four equal-sized cells and one small microcyte, and in ten other "tetrads" there were four equal-sized cells and one medium-sized microcyte. Of the forty "tetrads" counted, there were thirty-four which had four equal-sized cells, of which twenty-nine had additional cells.

TABLE II

FREQUENCY OF CELLS PER "TETRAD" FOR TWO TETRAPLOIDS

Number of cells per "tetrad"												
	1	2	3	4	5	6	7	8	9	10	11	12
Tetraploid A	0	0	0	7	10	13	7	1	1	0	1	0
Tetraploid C	0	0	0	5	20	6	8	1	0	0	0	40

In Tetraploid A there were twenty-nine 'tetrads' which contained four equal-sized cells, of which twenty-two had additional cells.

The 'tetrads' which contained large numbers of cells did not appear to have any orderly arrangement of cells within the 'tetrad,' but instead, resembled a sack of marbles. The largest number of cells contained in a 'tetrad' of the tetraploid was fourteen.

A closer examination of the tetrads of diploid anthers revealed that an occasional microcyte was present in the tetrads. They were so obscured as to pass unnoticed unless one was actually looking for them.

Some preliminary investigations of the seed from the two tetraploid plants have shown that Tetraploid A had a germination of 90 percent, and Tetraploid C had a germination of 20 percent.

In Tetraploid A, root tips were examined, and in four, where mitotic figures were observed, they were all diploid. The Tetraploid C root tips were examined and counts were made in two, both of which were tetraploid.

Discussion

There appears to be no significant cytological difference between lines of spinach showing different degrees of sexual expression. No evidence of the presence of a heteromorphic pair of chromosomes was found in any line. This, as noted previously, need only mean that the difference between the X and Y chromosomes is not of a nature or extent which is morphologically distinguishable.

The consistency of the 1:1 sex distribution, as well as more detailed genetic data presented below, indicates the presence of some form of segregation mechanism equivalent to the standard X-Y system.

It appears that the abnormal "tetrads" are the results of chromosomes which are separated from the main masses of Telophase II chromosomes. Telophase II proceeds regularly, but before cytokinesis is initiated, the chromosomes appear to spread out. Then cytokinesis becomes irregular, and actually appears to cut off certain numbers of the chromosomes to form microcytes.

PLATE I

MEIOSIS IN DIPLOID SPINACIA OLERACEA L.

1. Pachytene--(line C-1 intersex).
2. Diakinesis--(line A-10 male). This shows the characteristic forms of the chromosomes, and the two nucleolar chromosomes.
3. Diakinesis--(line A-10 male). This shows the two nucleolar chromosomes attached to the nucleolus after they have been forced out of the cells. All of the nucleoli shown have two attached chromosomes.
4. Late Diakinesis--(line A-10 male). The chromosomes are reduced to near Metaphase I size. The nucleolus is prominent and shows the two attached chromosomes.
5. Metaphase I--(line A-10 male). The chromosome number of twelve is readily determined. There appears to be no evidence for the presence of a heteromorphic chromosome pair.
6. Telophase I--(line C-1 intersex). The chromosomes completely lose their identity and Metaphase II follows without delay.
7. Telophase II--(line A-10 male).
8. Tetrads--(line A-10 male). The four groups of Telophase II chromosomes are cut off into individual microspores. The heteropycnotic regions remain visible in the microspores.
9. Pollen--(diploid intersex). They are uniform, and about 7 to 8 microns in diameter.

Scale--One division is equal to ten microns.

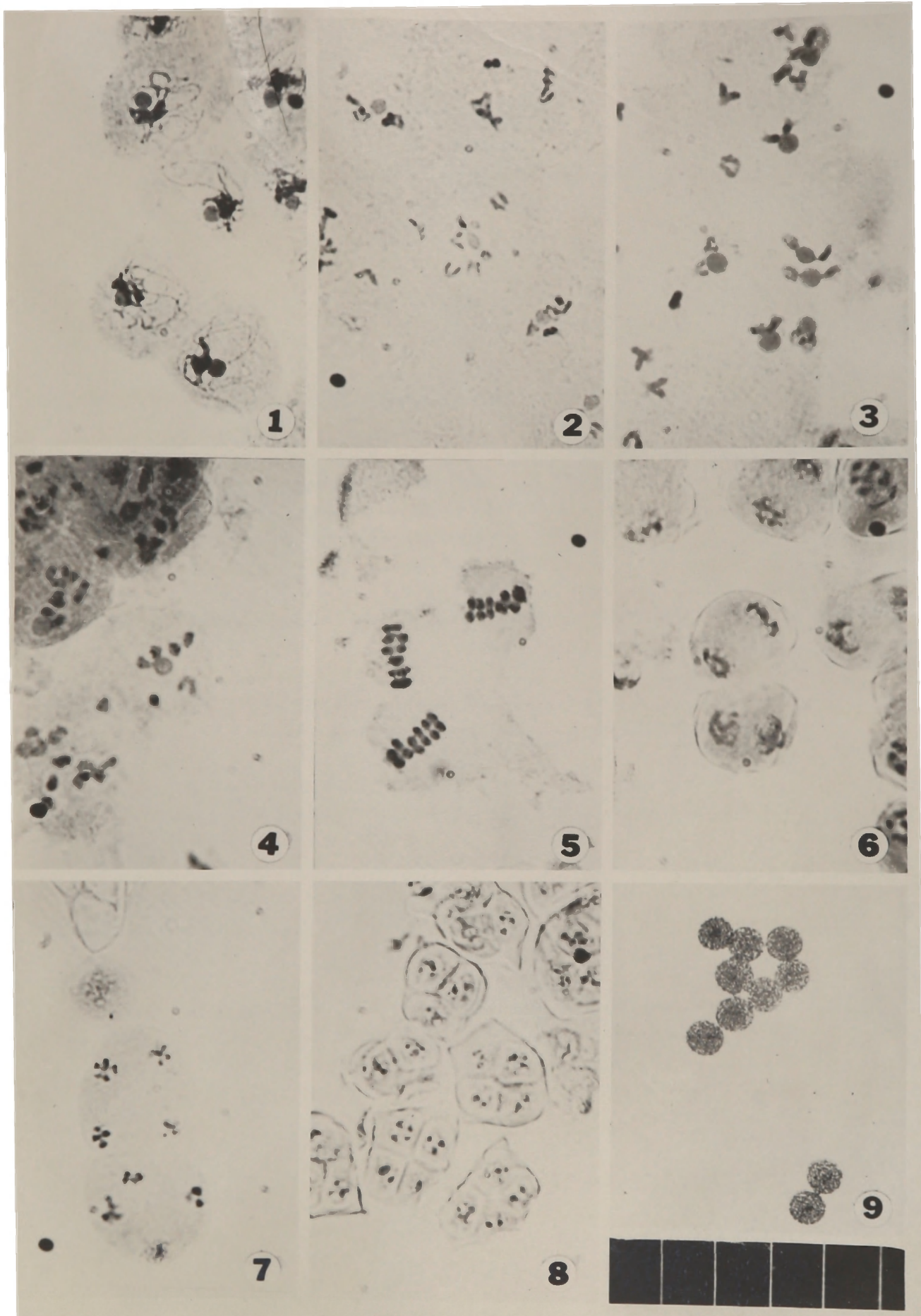


PLATE II

MEIOSIS IN TETRAPLOID SPINACIA OLERACEA L.
(all figures from Tetraploid A intersex)

1. Pachytene.
2. Diakinesis.
3. Metaphase I--At least one trivalent is clearly visible in the center of the group.
4. Anaphase I--The twenty-four chromosomes at either pole can be accurately counted at this stage. Note the three "lagging" chromosomes that appear to remain on the metaphase plate.
5. Telophase II--This shows the normal tight grouping of chromosomes at the four poles.
6. Telophase II--The chromosomes appear to spread out from the compact grouping of Mid-telophase II.
7. Cytokinesis stimulation after Telophase II--After the Telophase II chromosomes spread out, irregular cytokinesis is initiated.
8. "Polyads"--The result of the irregular cytokinesis is the formation of multicelled "tetrads." They vary in size and number per pollen mother cell.
9. Pollen--The irregular-sized pollen grains result from the abnormal "tetrads." The smaller pollen grains seems to be optically void of substance when stained with aceto-carmine.

Scale--One division is equal to ten microns.

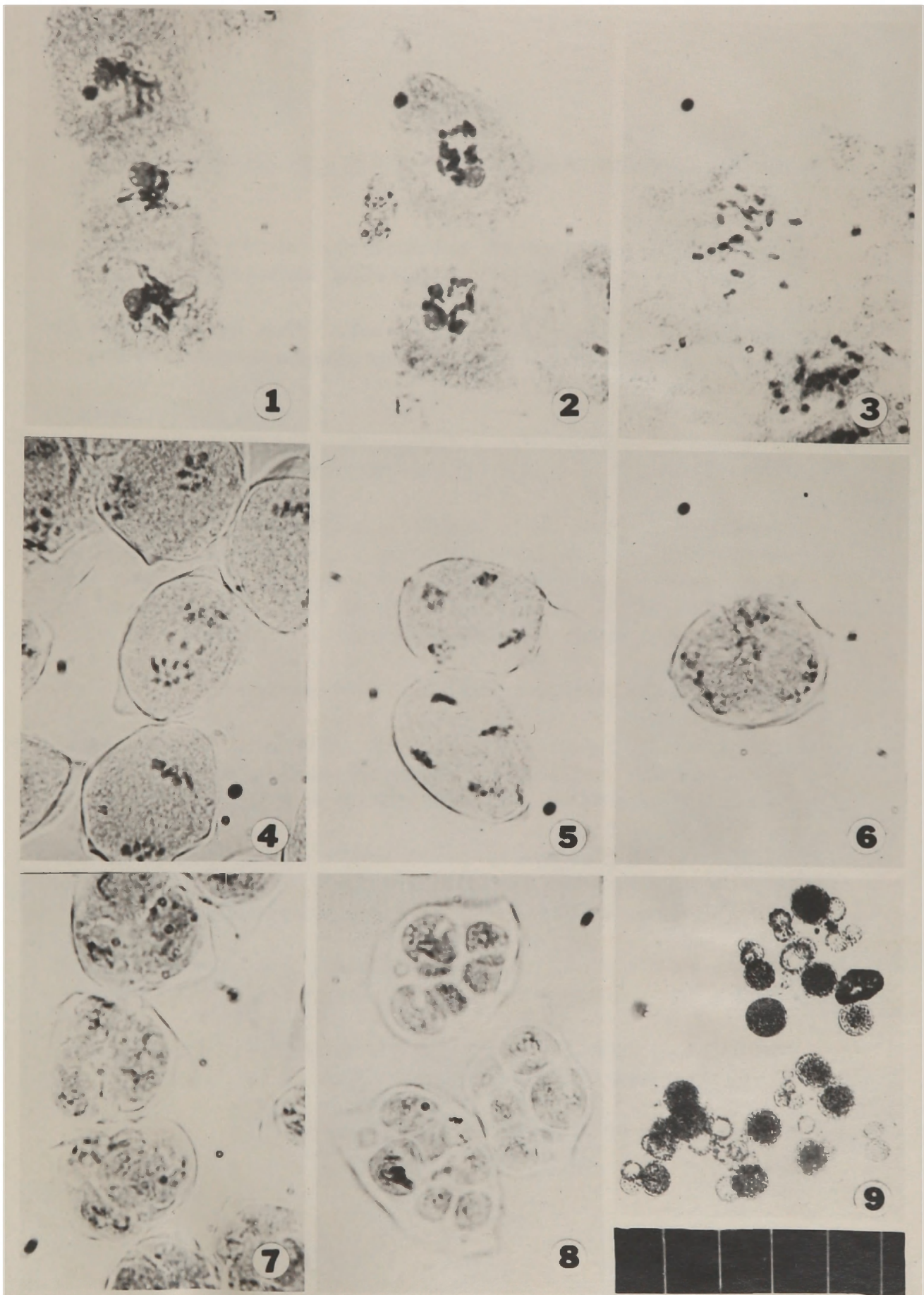
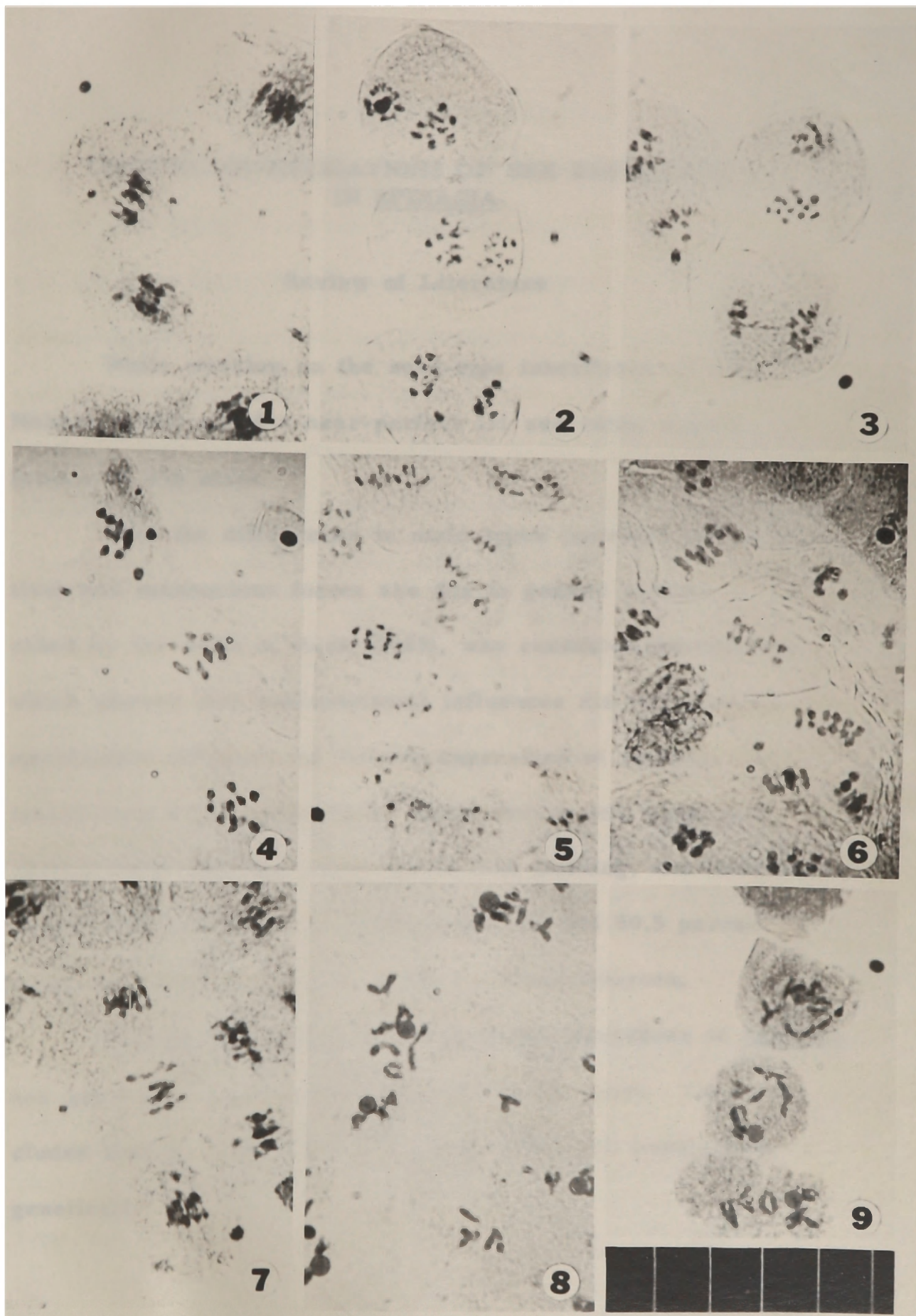


PLATE III

MEIOTIC CONFIGURATIONS IN SPINACIA OLERACEA L.

1. Metaphase I--(Tetraploid A intersex). Shows premature migration toward the poles, or possibly univalents.
2. Anaphase I--(Tetraploid A intersex). This stage shows the accurate counts of the twenty-four chromosomes, twelve chromosomes at each pole.
3. Anaphase I--(Tetraploid A intersex). Shows a chromosome bridge which is common in the tetraploid.
4. Metaphase I--(line L-15 intersex). The chromosomes are separating prior to the migration to the poles. Differences in the rate of separation are noted, and chromosomes with submedian kinetochores can be identified. There is no visible heteromorphic chromosome pair.
5. Metaphase II-Anaphase II--(line A-10 male).
6. Metaphase I--(line L-15 intersex). Shows no visible heteromorphic pair, and appears to be no different from the Metaphase I of the line A-10 male (Plate I, No. 5).
7. Metaphase I--(Tetraploid A intersex). This shows the presence of at least two distinct trivalents, in line on either side of the meiotic configuration in the center cell.
8. Diakinesis--(line A-10 male). From their characteristic shapes, the chromosomes can be identified at this stage.
9. Diakinesis--(line C-1 intersex). This line is different in having only one nucleolar chromosome pair.

Scale--One division is equal to ten microns.



GENETIC INVESTIGATIONS OF SEX EXPRESSION IN SPINACIA

Review of Literature

While working on the seed-type inheritance of spinach, Nohara (1923) noted a near-perfect 1:1 sex ratio; namely, 495 female to 496 male.

That the differences in male types (extreme and vegetative) and monoecious forms are due to genetic factors is indicated by the work of Rosa (1925), who conducted experiments which showed that environmental influences did not have any appreciable influence on the sex expression of spinach. He tested rich versus poor soils, shade versus full light, wide versus close spacing, early versus late planting, and mutilation. Of his total population of 5,198 plants, he had 50.5 percent male, 49.3 percent female, and 0.2 percent intersex.

Hirata and Yamamoto (1930) selfed intersexes of Spinacia and grew only females and intersexes from them. They concluded that the intersexes they dealt with were female-like, genetically.

Negodi (1934) investigated sex inheritance in S. oleracea, and suggested that sex was controlled by a multiple complex of genes constituting a sexual group, the female being homogametic and the male being heterogametic. He suggested that the monoecious condition depended on differences in the female complex, and either would breed true or segregate into monoecious and female. Environment was considered to be a factor in the development of a certain type of monoecious condition. His scheme was of the type where $MMFF$ = female, $MMFf$ = male, with F_1 , F_2 monoecious factors. $MMFF_1$ = monoecious, which segregates 0.25 percent $MMFF$ being female, 50 percent $MMFF_1$ being like the parent, and 25 percent MMF_1F_1 being true breeding intersex.

MMF_1F_2 = monoecious which segregates 25 percent MMF_1F_1 being true breeding intersex, 50 percent MMF_1F_2 being like the parent, and 25 percent MMF_2F_2 environmental controlled monoecious, or female.

Miryuta (1937) determined that it was quite possible to obtain constant forms, producing female and monoecious plants in a 1:1 ratio, with complete consistency of the monoecious type, as regards sex characters. In the F_1 , it is possible to

obtain (1) male and monoecious (near male plant), (2) all monoecious plants with segregation into two monoecious subgroups, and (3) all monoecious plants without segregation. He suggested that sex types in S. oleracea are not controlled by environment, but that they come to full development only under certain environmental conditions.

Sugimoto (1947) offered a genetical explanation based upon an X-Y mechanism plus factors ZZ. He had six types of factors: (1) female factor--ZZXX; (2) environmental female intermediate--ZzXX; (3) male factor--zzXX; (4) environmental male intermediate--ZzXY; (5) female intersex--zzXX; and (6) male intersex--ZZXY.

From the translation of the paper, there was no apparent explanation for the selfing of male-type intersex plants which contained the XY factor. From a mathematical viewpoint, the selfing of an X-Y individual will result in one-fourth of the progeny having a YY factor.

Environment as a Factor

In the spring of 1950, before this present work was initiated, a trial planting of spinach, consisting of two varieties,

was planted to check an unpublished report of an experiment conducted by Bowser (1943), indicating that spacing and date of planting would influence the sex ratio. From the results of this trial planting (Table XVI in the Appendix), it was concluded that neither the spacing nor the date of planting significantly influenced the sex ratio.

Rosa (1925) pointed out that thinning would often influence the sex ratio, owing to the selection of the plants being thinned out. The male plants are often the smallest at the stage when thinning is practical, and the tendency to remove the smallest plants (mostly male) will cause the remaining population to be predominantly female or intersex.

An influence of date of planting on the sex ratio may be indicated unless the plants are all scored at the same age. The earlier plantings will often show an increase in the number of intersexes if compared with later plantings composed of younger plants. The intersex condition in specific plants will manifest itself to a greater degree as the plant matures. This is true only of plants of a specific genetic make-up, a version of Sugimoto's "environment intermediate factor."

Table III contains the total numbers of male, female, and intersex plants from the trial planting in 1950.

When this was broken down by varieties, the striking difference was in the number of intersexes in each variety-- 8.3 percent, compared to 1.9 percent. Inasmuch as environmental conditions were similar for each variety, this difference may be attributed to genetic factors. A program was initiated to test this contention.

Materials and Methods

Intersex plants which were open-pollinated were selected from this 1950 spring planting, and a breeding program was initiated. By utilizing the greenhouse, four complete generations were produced and evaluated. In the winter of 1950-51, fifteen progenies were grown; twelve individuals from seven of these progenies were selected and planted in the field in the spring of 1951. In the winter of 1951-52, six progenies were grown from one cross and five selfs selected from the 1951 spring plantings. In the spring of 1952, fifty progenies were grown from crosses and selfs of the material grown in the winter of

TABLE III
SEX EXPRESSION OF TWO VARIETIES OF SPINACH
(field planting)

Variety	Total		Male		Female		Intersex	
	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
Long Standing Bloomsdale	1,262	66.1	598	47.6	559	44.3	105	8.3
Nobel	648	33.9	310	47.8	326	50.3	12	1.9
Total	1,910	100.0	908	47.5	885	46.3	117	5.7

1951-52. Data from all plantings are given in Tables XII, XIII, XIV, XV, in the Appendix.

In addition to crossing and selfing diploid plants, the use of polyploidy was to be included. However, to date, only two tetraploid plants have been produced. As yet, no crosses have been attempted, pending further study of the stability of the tetraploid material as described in the cytological study.

Genetic Observations

For the most part, the data to be used in this genetical discussion have been taken from the spring crop grown in 1952. Spinach, because of its anemophilous nature and its predominantly dioecious condition, is extremely heterozygous. It has therefore taken four generations of controlled breeding to obtain lines which were pure enough to show genetical patterns of inheritance. This is demonstrated by the data collected on the color factor (A), which has now been shown to follow a single gene type of inheritance. But it was not until the fourth generation that this could be shown to be true.

One criticism of the previously presented schemes of sex inheritance is that the analysis has been worked out from data from a small number of plants, grown for only a few generations. Sex inheritance in spinach is obviously too complex to be explained from such a small amount of data. The present study, although more extensive than most, still provides data suitable only for a general analysis.

In formulating a genetic scheme of sex inheritance, the presence of an X-Y mechanism has been assumed. This is purely an assumption, based upon genetic data, and not from

cytological observations of "sex chromosomes." As previously mentioned, the presence of a heteromorphic chromosome pair has never been reported in S. oleracea, nor was the presence of such a pair detected in this study. However, as stated before, this is not conclusive evidence against the existence of a pair of chromosomes which act as "sex chromosomes." It is difficult to explain the 1:1 sex ratio without such a mechanism.

The presence of modifying sex genes located on a chromosome separate from the X and Y chromosomes was also assumed, based on genetic data.

The third assumption was the balancing of the modifying sex genes by some mechanism in order to maintain a 1:1 sex ratio under natural conditions. Commercial varieties of spinach have been selected for many years, toward the elimination of the extreme male type and toward an increasing number of intersex plants, until the mechanism for control of the 1:1 sex ratio has been altered. Dark Green Bloomsdale, Long Standing Bloomsdale, Juliana, Nobel, and Hollandia are examples of commercially important varieties that have originated from single plant selections of intersex plants. Even though these varieties have had this mechanism altered during their origination,

nevertheless, they still predominantly maintain the 1:1 sex ratio. The complete alteration of this mechanism has been accomplished only recently by the establishment of pure-breeding intersex lines.

A problem of major importance is the placing of sex types into classes. Females, males, and extreme male types are quite distinct in their morphology, and are easily recognized. However, the various degrees of intersex plants present a different situation. To place the intersex plants into a single classification makes the genetic interpretation difficult, and it is also difficult to determine the proper classification for the varying degree of intersex or to determine the extent of environmental influence on the intersex condition. By scoring the sex type of the plants at approximately the same stage of development, the varying environmental influences are held to a minimum. In this study, seven classifications for sex type have been designated.

Plant types.

1. Female--At an early stage, flower development is characterized by long stigmas, and as the plant matures and pollen is available to bring about fertilization, the rapid

development of seed takes place. Points to check are the terminal ends of the lateral branches and the base of the main flower stalk. It is in these areas where hermaphroditic flowers appear, if the plant is a female intersex.

2. Female intersex--The plant, if young, may pass as female, but as it approaches maturity, approximately 30 percent of the flowers are either hermaphroditic or androecious. The greatest concentrations of these flowers are usually near the terminals of the flower stalks.

3. Intersex--This type of plant produces mainly androecious and gynoecious flowers in approximately equal numbers, with a smaller number of hermaphroditic flowers. The intersex expression is visible with the first flower clusters.

4. Male intersex--This type is identified by having a majority of androecious flowers. In extremes of this type only a few functional ovaries are present. These are usually found near the central portion of the main flower stalk.

5. Nonfunctional male intersex--This type may easily be scored as pure male, as no functional ovaries are present. In apparently androecious flower buds, small hair-like stigmas protrude from the center of the flower before the stamen

filaments elongate. The number of these nonfunctional hermaphrodite flowers is usually few, and they can easily be missed. Care must be taken not to confuse the withered stamen filaments of old flowers for stigmas, as there is a close resemblance between the two. This type of plant is morphologically male in appearance, being smaller and maturing earlier than females or functional intersexes. It is possible that the gynoeceious expression in this type of plant is comparable to the androeceious expression in the strong female intersex, except that in reducing the androeceious expression, it is the number of anthers which are reduced, and in the case of the gynoeceious expression the ovary becomes reduced to a nonfunctional body. However, one functional anther may produce enough pollen to fertilize a great number of gynoeceious flowers.

6. Vegetative male--The flowers on this type are purely androeceious, and the plant resembles the nonfunctional male intersex type.

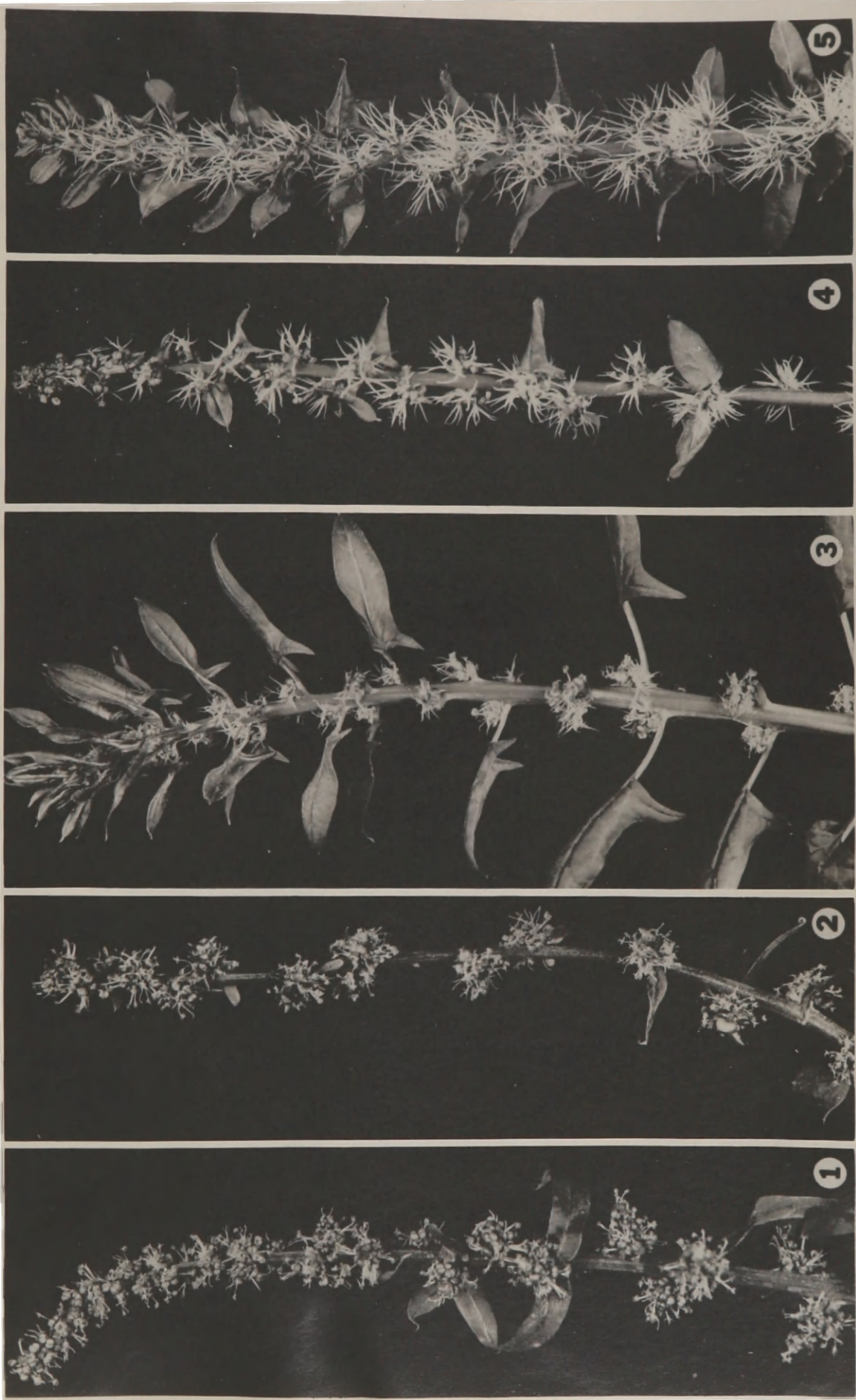
7. Extreme male--This type is morphologically different in that it flowers early, seldom branches, and matures and dies early. The flowers are all androeceious. However, there have been hermaphroditic flowers noted on the extreme male-type

PLATE IV

SEX TYPES IN SPINACIA OLERACEA L.

1. Extreme male type--The flowers are all androecious. The stamen filaments, after the anthers have dehisced and dried up, may give the appearance of stigmas.
2. Male intersex--Note the horned-type seeds, which are limited to one or two per flower cluster.
3. Intersex--Androecious and gynoecious flowers are about equal in number throughout the plant.
4. Female intersex--The plant was predominantly gynoecious, but the terminal branches as shown here reverted to the androecious condition.
5. Female--The flowers are all gynoecious; note the length of the stigmas.

These figures also indicate that flower clusters containing gynoecious flowers are more apt to be accompanied by small leaves than flower clusters containing androecious flowers.



plant in certain foreign strains of spinach grown here at this station.

Flower types. Seven flower types which show five intergradations from gynoecious to androecious flowers are indicated by seven drawings in Figure B. The drawings were made from flowers which were dissected from a single female intersex flower stalk. Visually, morphologically pistillate flowers (Type 1) are characterized by their long-branched stigmas, the length of which varies considerably among different plants. The ovary is large and increases in size as soon as fertilization takes place. Perfect (Type 2) is a weak androecious type flower, but the single anther produced is functional, producing a considerable amount of pollen. The appearance of the anther usually is late, and often the flower has been fertilized before the included anther matures. Perfect (Type 3) is functional for both parts. The number of branches of the stigma is usually reduced, but the ovary is still large. Often small bracts are formed near the anthers. Perfect (Type 4) is often functionally staminate only, as the ovary is considerably reduced, and often aborts, rather than forming normal seed. The naked seed (ovule) often grows out of the ovary, and is not fertile. Types 5 and 6 are

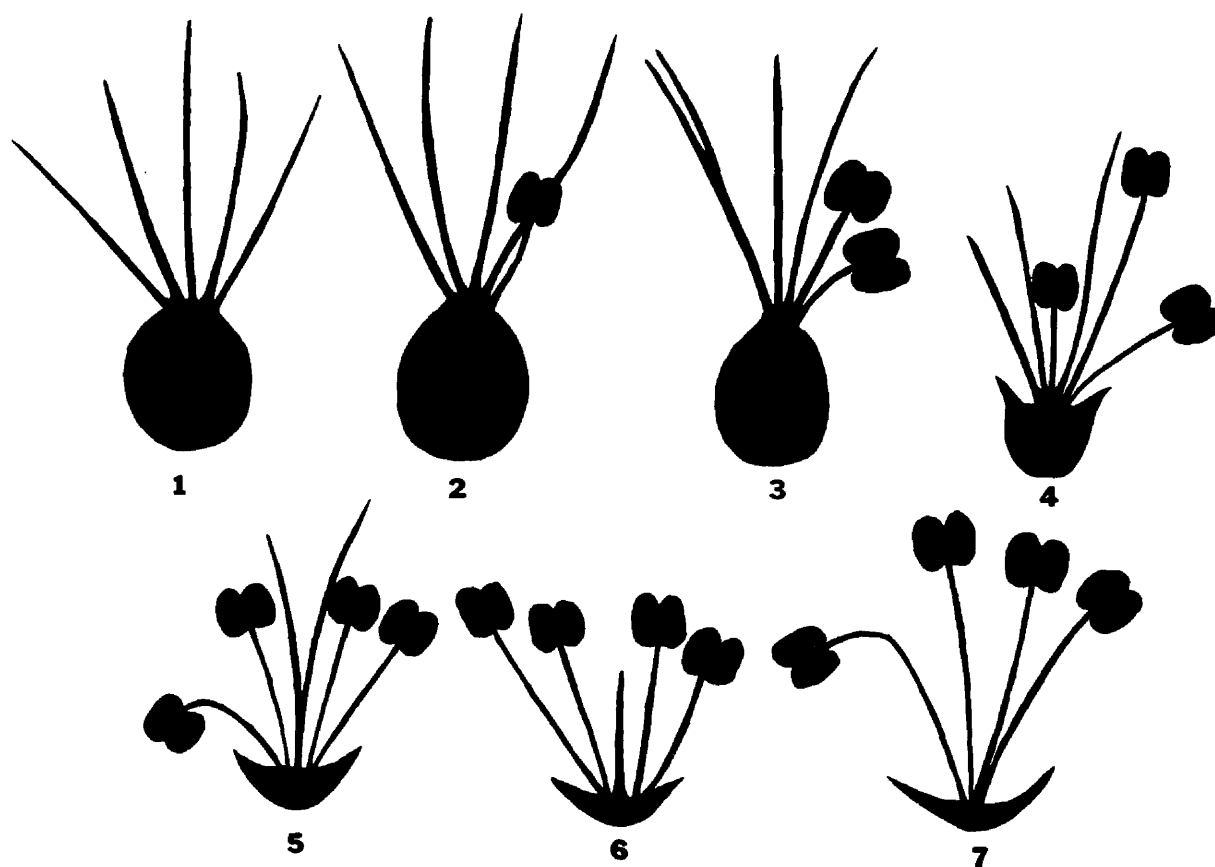


FIGURE B

FLOWER TYPES

1. Visually morphologically pistillate (Type 1).
2. Perfect (Type 2).
3. Perfect (Type 3).
4. Perfect (Type 4).
5. Functionally staminate (Type 5).
6. Functionally staminate (Type 6).
7. Visually morphologically pistillate (Type 7).

functionally staminate flowers. Rudimentary stigmas are formed and may be branched and extend beyond the anthers, or may be a single hair-like structure which extends beyond the anthers only, while they are in the bud stage. These types of flowers are often confused with pure androecious flowers which have matured their anthers. The stamen filaments shrink in size and the anther wall falls off, giving the appearance of stigma-like structures. The bud stage is the best time to examine for functionally staminate flowers. Visually morphologically staminate flowers (Type 7) usually contain four anthers, but this number is not constant. Occasionally, after the flower has matured and the stamen filaments have shrunk to the size of stigma-like structures, the large bracts will fold upward and can give an appearance similar to that of a pistillate flower.

Only by using such a system of classification can the minimum number of factors required to fit a genotype to the various classes be determined.

The ZZXY system, as proposed by Sugimoto (1947), is based upon a scheme similar to the type used here, but his is limited in the number of classes to which his factors will fit;

however, his term "environmental intermediate" allows the inclusion of several described types.

A scheme based upon crossing over between the X and Y chromosomes, which permits varying amounts of each sex-determining chromosome to be included, adapts very well to the varying degree of the intersex condition, and to the segregation of either females or males, but breaks down when selfed intersex plants segregate into males and females with or without the intersex condition also segregating.

From data obtained with the breeding material of spinach used in this study, an acceptable genetical scheme for sex inheritance must explain:

1. Constant 1:1 sex ratio.
2. Production of intersexes of different degrees.
3. Maintenance of a pure-breeding intersex line.
4. Segregation of intersexes into (a) males, females, and intersexes; (b) females and intersexes only; or (c) males and intersexes only.
5. Pure sex-intersex crosses of different types.

Genetic Explanation

The genetic scheme devised to fit the conditions of the observed results of the breeding material is shown diagrammatically in Figure C. It has been arranged in a modified Punnett square to show the gametes and the resulting genotype of the individuals, and by keying out to the margin it shows the gametes produced by each type of individual, assuming complete linkage of the A-G genes. The A and G genes are the androecious- and gynoecious-determining genes.

By assuming a very low crossover value, nearly complete coupling linkage of the A-G genes, then the great majority of genotypes produced would be as represented by the eight corner squares of the XX and XY blocks. This system will then maintain a 1:1 ratio for sex.

In order to produce the intersex condition there must be crossing over between the A-G genes, and this must naturally occur in individuals of the (Aa Gg) type. This condition also reduces the chances for the formation of intersex plants, as crossing over in the (AA GG) or (aa gg) individuals will not alter the genotype of the gametes produced. In fact, it might be possible to have the A-G genes present in only the recessive,

EXPLANATION FOR FIGURE C

The XX block and the YY block segregate only two types of gametes, while the XY block segregates four types of gametes, as indicated in the margins. In all cases the A-G genes are assumed to be linked with a very low crossover value. However, they are independent of the X or Y chromosomes.

$AA > Aa > aa$, for androecious tendencies, and likewise, $GG > Gg > gg$, for gynoecious tendencies.

FIGURE C

DIAGRAMATIC SCHEME FOR THE GENETIC EXPLANATION
FOR SEX INHERITANCE IN SPINACIA

Gametes	X A-G	X A-g	X a-G	X a-g
X A-G	XX AA GG	XX AA Gg	XX Aa GG	XX Aa Gg
X A-g	XX AA Gg	XX AA gg	XX Aa Gg	XX Aa gg *
X a-G	XX Aa GG	XX Aa Gg	XX aa GG	XX aa Gg
X a-g	XX Aa Gg	XX Aa gg *	XX aa Gg	XX aa gg

* Denotes environmentally unstable intersex types.

FIGURE C (Continued)

Gametes	X A-G	X A-g	X a-G	X a-g	Gametes
X A-G	XY AA GG	XY AA Gg	XY Aa GG	XY Aa Gg	Y A-G
X A-g	XY AA Gg	XY AA gg	XY Aa Gg	XY Aa gg	Y A-g
X a-G	XY Aa GG	XY Aa Gg	XY aa GG	XY aa Gg *	Y a-G
X a-g	XY Aa Gg	XY Aa gg	XY aa Gg *	XY aa gg	Y a-g
Gametes	Y A-G	Y A-g	Y a-G	Y a-g	Gametes

* Denotes environmentally unstable intersex types.

FIGURE C (Continued)

Gametes	Y A-G	Y A-g	Y a-G	Y a-g
Y A-G	YY AA GG	YY AA Gg	YY Aa GG *	YY Aa Gg
Y A-g	YY AA Gg	YY AA gg	YY Aa Gg	YY Aa gg
Y a-G	YY Aa GG *	YY Aa Gg	YY aa GG	YY aa Gg *
Y a-g	YY Aa Gg	YY Aa gg	YY aa Gg *	YY aa gg

* Denotes environmentally unstable intersex types.

or dominant condition in the population, which would eliminate the possibility of the production of intersexes, and maintain a constant 1:1 sex ratio.

Assuming some crossing over does take place (all homologous chromosome pairs were observed to show chiasmata formation during meiosis in S. oleracea), the first step is the production of pure-sex individuals which will produce gametes which, when combined, will give a large proportion of intersex types, or it is possible to produce intersex types directly as the result of the crossing-over. For example, the individual (XX Aa Gg) will, as a result of crossing over, produce gametes of the (X A-g) and X a-G) type. These gametes combined with Y-containing gametes will produce males of the type (XY A- g-), or male intersexes of the type XY a- G-), and if combined with X-containing gametes they will be reversed in their action and produce females of the (XX a- G-) type, or female intersexes of the type (XX A- g-).

The female intersexes produced in this manner would also be of two types. The "strongest" intersex of the type (XX AA Gg) would, when selfed, segregate into females, the paternal type, and into the pure-breeding intersex type, in the

ratio of 1:2:1. The other female intersex of the type (XX Aa gg) would be a strong female intersex, subject to environmental influences to a greater extent, and would also segregate, when selfed, into females, the parental type, and into the pure-breeding intersex type in the ratio of 1:2:1.

To meet the conditions of the segregation of selfed intersex plants into males, as well as females and intersex types, the YY type of individual was considered to be viable, and to develop normally.

From the cytological study where the twelve chromosomes were of six morphologically identical pairs, it would seem plausible that there were no great differences between the chromosomes making up the X-Y mechanism. It would also then seem plausible that the existence of a YY individual that would differ only in its degree of sex expression would be possible. The genetical data bear this out, as will be shown. Assuming this condition to exist, the XY male intersex plants will then segregate when selfed into females, the parental type, other intersex types, and males, depending upon the genotype of the parent intersex.

In the case where only males and male intersexes segregate from a selfed male intersex, the absence of the X chromosome must be assumed. In other words, the male intersex was of the YY type.

From the genetical scheme involving the X-Y mechanism and the A-G modifying genes, there are twenty-seven genotypes (or thirty, counting linkage patterns) representing six phenotypes. They are as follows:

I. Males (pure breeding, environmentally stable).

(XY AA GG)	
(XY Aa Gg)	(A-G, a-g linked)
(XY aa gg)	
(YY AA GG)	
(YY Aa Gg)	(A-G, a-g linked)
(YY aa gg)	

Note: The YY males are uncommon, being the result of selfed XY intersex plants, or such intersex plants outcrossed to XY males, and the YY males will in turn produce only males when crossed to female plants.

II. Males (segregating, environmentally stable).

(XY AA gg)	
(XY Aa gg)	
(XY AA Gg)	
(XY Aa Gg)	(A-g, a-G linked)

(YY AA gg)
 (YY Aa gg)
 (YY AA Gg)
 (YY Aa Gg) (A-g, a-G linked)

Note: This type of YY male, when crossed to female plants, will give Type II XY males and XY intersex plants.

- III. Nonfunctional male intersex (segregating, environmentally influenced to produce seed).

(YY Aa GG)
 (YY aa Gg)

- IV. Male intersex (segregating, environmentally unstable).

(XY Aa GG)
 (XY aa Gg)

- V. Male intersex (pure breeding, fair degree of environmental stability).

(YY aa GG)

- VI. Intersex (segregating, high degree of environmental stability).

(XY aa GG)

- VII. Intersex (pure breeding, environmentally stable).

(XX AA gg)

- VIII. Female intersex (segregating, environmentally unstable).

(XX Aa gg)
 (XX AA Gg)

IX. Female (segregating, environmentally stable).

(XX Aa GG)
 (XX aa GG)
 (XX Aa Gg) (A-g, a-G linked)
 (XX aa Gg)

X. Female (pure breeding, environmentally stable).

(XX AA GG)
 (XX Aa Gg) (A-G, a-g linked)
 (XX aa gg)

The six phenotypes represented and identifiable, barring environmental influences, are males, nonfunctional male intersexes, male intersexes, intersexes, female intersexes, and females.

The morphologically extreme male is no different in sex from the vegetative males.

The complete data for all plantings are included in the Appendix, and only specific cases will be used here to illustrate particular modes of inheritance.

The formation of a pure-breeding intersex line is best shown by the progeny of the ten selfed intersex plants of the pedigree (XF-14-11-15) which were grown in the spring of 1952.

The XF plant was a male intersex type selected from a planting of Long Standing Bloomsdale grown in the spring of

1950. This plant was open-pollinated, but was selfed to a high degree by the heavy production of androecious flowers. In the greenhouse in 1950-51, its progeny segregated into six male, four female, and four intersex plants. The four intersex plants were selfed and planted in the field in 1951. Line (XF-14-11), which was the progeny of one of these intersex lines, segregated into three male, three female, eight female intersex, twenty-one intersex, and twelve male intersex plants. Two intersex plants were selected from this progeny, selfed, and grown in the greenhouse in 1951-52. From the progeny of one of these plants (XF-14-11-15), ten intersex plants were selfed and planted in the field in 1952. The pattern of segregation of these is shown in Table IV.

It is quite obvious that this particular line is breeding true for the intersex condition. The variations noted are probably due to environmental influences on the rate of maturation of the plants, and to some slight error in scoring. The genotype of this line may be considered as being (XX AA gg), and should remain as a true breeding line for the intersex condition.

Examples of selfed intersex plants segregating into females and intersexes are indicated by the data for four progenies shown in Table V. One line was grown during the spring of 1951, and three, during the spring of 1952.

TABLE IV
PURE-BREEDING INTERSEX TYPE (XX AA gg)

Pedigree No.	Male Intersex	Intersex	Female Intersex
(XF-14-11-15-1)	1	45	3
(XF-14-11-15-2)	10	68	-
(XF-14-11-15-3)	7	33	1
(XF-14-11-15-4)	2	55	1
(XF-14-11-15-5)	20	56	2
(XF-14-11-15-6)	-	110	2
(XF-14-11-15-7)	3	73	4
(XF-14-11-15-8)	3	53	5
(XF-14-11-15-9)	20	36	1
(XF-14-11-15-18)	14	36	-

TABLE V
SELFED INTERSEX SEGREGATING INTO FEMALES AND INTERSEXES

Pedigree No.	Observed		Expected		χ^2	P Value
	Intersex*	Females	Intersex*	Females		
(XF-14-9)	19	25	33	11	-	-
(XF-14-11-7-22)	26 + 52**	32	27.5 + 55***	27.5	0.981	0.65
(XF-14-12-8-36)	24	21	33.75	11.25	-	-
(XF-14-12-8-37)	48	13	45.75	15.25	0.442	0.50

* Includes all intersex types.

** Denotes 26 plus 52 male intersex types.

*** Denotes 27.5 plus 55 male intersex types.

Number (XF-14-9) was in the second generation of selfing, and probably was still heterozygous to a high degree, but showed the pattern toward female and intersex types.

For number (XF-14-11-7-22) the 1:2:1 ratio fits the data giving a chi-square value of 0.981 and a P value of 0.65. The genotype fitting this case would be (XX Aa gg), which segregated 1 (XX AA gg) : 2 (XX Aa gg), which are both intersexes, and 1 (XX aa gg), which is female. The differences in types of intersexes again may be attributed to environment and scoring. The important factor, however, is that here are three intersex types to one female type.

Number (XF-14-12-8-36) is a poor 3:1 fit, but the general pattern is there. In number (XF-14-12-8-37), the chi-square value for a 3:1 ratio of intersex types to females is 0.442, and a P value of 0.50, and the same genotype would fit as in the case with number (XF-14-11-7-22).

Cases of selfed intersex plants segregating into males and intersex types are shown from the progeny segregation of two intersex plants in Table VI. Number (XF-14-12) fits a 3:1, with a chi-square value of 0.785 and a P value of 0.40 for the ratio of one male to three intersex-type plants.

TABLE VI
SELFED INTERSEX SEGREGATING INTO MALES AND INTERSEXES

Pedigree No.	Observed		Expected		χ^2	P Value
	Males	Intersex*	Males	Intersex*		
(XF-14-12)	24	58	20.5	61.5	0.785	0.40
(XF-14-12-8-38)	6	35**- 41				

* Includes all intersex types.

** Denotes nonfunctional male intersex type.

The genotype of (YY aa Gg) segregates into 1 (YY aa gg) male, 2 (YY aa Gg) parental-type intersex, and 1 (YY aa GG), which is considered as a strong male intersex type.

The second example of this type appears to be breeding true for the male intersex type, allowing for environmental and scoring variations. The genotype (YY aa GG) would satisfy the requirements in this case.

Further evidence for the XY intersex and YY-type individuals is shown in the data from line [(XF-3-2) x (XF-3-2)]-1, which was a brother-sister cross, with intersex plants from this progeny being selfed, and their progeny grown in the field in 1952.

The individual (XF-3-2) was an intersex plant selected from a segregating population of seventeen males, nine females, and two intersex plants. The progeny from the selfing of this plant was grown in the field in 1951. It segregated into forty-one males, twelve intersexes, and twenty-two females.

The theoretical ratio for the segregation of an (XY aa Gg) intersex would be four female, three male, three nonfunctional male intersex, four parental type, and two intersex. If we group the males and male intersex, including parental type,

into the male class, then a ratio of four female, two intersex, and ten male plants is expected.

This grouping of male intersexes with males, including the parental type, is suggested because of the influence environment has on sex expression of the intersexes. Observations indicate that in greenhouse generations, the androecious expression is delayed, while the gynoecious expression persists. It was then possible to have a male-type intersex express itself and set a large amount of seed, while the same genotype in the field could possibly be scored as a male or nonfunctional male intersex.

The ratio of 4:2:10 fits the data, with a chi-square value of 1.971 and a P value of 0.40.

Unfortunately, the segregation of the progeny from this cross, which was grown in the greenhouse, was not obtained because the plants were scored and removed before the complete expression of their sex was manifested. However, from this progeny, eleven intersex plants were selfed and their progeny grown in the field in 1952. By grouping the males and non-functional male intersexes into one class, the remaining intersex

types into another class, and females in the third class, the following ratios were obtained, and are shown in Table VII.

Considering only the XY segregation, and assuming the modifying genes to be strongly androecious, the expected distribution is 1 XX : 2 XY : 1 YY, or one female to three male or intersex. This 3:1 ratio was applied to the data, and the chi-square and P values are indicated in Table VII.

The segregation of males and intersex types was not quite as conclusive, but here again the sex balance was approaching equality and was subject to environmental influences to a greater degree. However, plants (by using the last number in the pedigree to designate plants) numbered 47 and 49, with the high proportion of intersexes, are probably of the genotype (XY aa GG), which segregates one female to three intersex. Also, plants numbered 43, 44, 45, 46, and 48 appear to be fairly strong for the intersex condition, and are probably of the genotype (XY aa Gg). The remaining four plants, numbered 40, 41, 42, and 50, appear to be strongly male, and are probably of the genotype (XY Aa GG).

TABLE VII

SELFED INTERSEX SEGREGATING INTO MALES, INTERSEXES, AND FEMALES

Pedigree No.	Male*	Observed		Expected		χ^2	P Value
		Intersex**	Female	Male* + Intersex**	Female		
(XF-3-2)	41	12	22	(46.8 + 9.4)	18.8	1.971	0.40
(---40)***	46	9	20	56.25	18.75	0.111	0.73
(---41)***	67	8	24	72	24	0.498	0.49
(---42)***	91	-	24	86.25	28.75	1.047	0.31
(---43)***	60	20	21	75.75	25.25	0.993	0.32
(---44)***	31	22	18	53.25	17.75	0.005	0.94
(---45)***	48	21	21	67.5	22.5	0.133	0.72
(---46)***	39	33	24	72	24	0.000	0.99
(---47)***	9	43	19	53.25	17.75	0.117	0.73

TABLE VII (Continued)

Pedigree No.	Male*	Observed		Expected		χ^2	P Value
		Intersex**	Female	Male* + Intersex**	Female		
(---48)***	25	32	25	61.5	20.5	1.346	0.25
(---49)***	14	30	19	47.25	15.75	0.896	0.35
(---50)***	62	3	19	63	21	0.254	0.60

* Includes nonfunctional male intersex.

** Includes all other intersex types.

*** Complete pedigree includes (XF-3-2) x (XF-3-2) - 1 - (number used in the table).
Example: (---40) is actually (XF-3-2) x (XF-3-2) - 1 - 40.

This follows the pattern of production of these types, which are produced in the ratio of 2:1:2; that is 2 (XY Aa GG) : 1 (XY aa GG) : 2 (XY aa Gg).

I. The Cross Between Intersex and Strong Male. The strong male was taken from an increase planting of a foreign plant introduction population (F.P.I. 164965). The plant was of the morphologically extreme male type.

This cross was accomplished in the greenhouse in 1951-52, as were the other crosses. As stated before, the androecious condition is suppressed or delayed in greenhouse planting, and because of this condition the intersex plant had produced no androecious flowers, but was in a full gynoecious bloom. These gynoecious flowers were pollinated with pollen from the extreme male plant and the seed had set before any androecious flowers appeared. The plant was then pruned back, leaving only the portion of the stems bearing fertilized seeds. The androecious flowers then appeared, but as no more gynoecious flowers developed, there was no danger of producing selfed seed. The progeny bore this out by using other factors as controls. In this case it was the horned-seed condition which was dominant, and the male plant was homozygous for this dominant character;

thus, all seed-bearing plants in the progeny expressed this horned character.

The results of this cross as to sex expression were thirty-nine morphologically extreme males, twenty-three intersexes, and sixteen female intersexes. This is a perfect one male to one intersex. The genotype of the male was probably (XY Aa Gg), with A-G and a-g linked. The intersex genotype could have been (XX AA gg). This cross would then segregate 1 (XY AA Gg) : 1 (XY Aa gg), both being males, and 1 (XX AA gg) intersex : 1 (XX Aa gg), which should show the female intersex condition. This scheme fitted the data very well.

II. Female Crossed With Morphologically Extreme Male (F.P.I. 164965). The progeny segregated fifty-three morphologically extreme males, forty-one females, and no intersexes. The male was (XY Aa Gg), as indicated in the first cross, and the female could be any one of three types: (XX AA GG), (XX Aa Gg), or (XX aa gg). By assuming linkage of the A-G or a-g, only males and females would result. The ratio (53:41) has a chi-square value of 1.532 and a P value of 0.21 for a 1:1 ratio.

III. Female Crossed With Morphologically Extreme Male (F.P.I. 164965). The results for sex expression were thirty-one

morphologically extreme males and twenty-six females, plus six strong female intersex plants. The male was (XY Aa Gg), and in this case the female was probably (XX Aa gg). This could be possible because this female was from the progeny of a selfed intersex plant. The expected results of such a cross would be two males to one female to one female intersex of two types.

Again, environment could modify the 1:1 ratio of female to female intersex type. But, the fact that some androeciousness was present in the females in this cross, and not in the previous cross, indicated a difference in the genotype of the two female plants involved.

IV. Female Crossed with Intersex Plant. The intersex plant used was a pure-breeding intersex, the progeny of which gave one hundred ten intersex and two female intersex plants. It was then of the genotype (XX AA gg). This plant, when used as the pollen parent for a female plant, gave a progeny of nine intersex, eighty-four female intersex, and four female. The nine intersex and four female plants observed could have been due to environmental influences or errors in scoring.

Assuming a female with the genotype of (XX AA GG), then the resulting progeny would be all (XX AA Gg), which is a female intersex genotype.

V. Female Crossed With Intersex Plant. The intersex plant was of the genotype (XY Aa GG), as indicated by the ratios of its progeny as a result of having been selfed (Refer to Plant No. 40). The segregation for sex expression of this cross was one male, twelve nonfunctional male intersex, twenty-nine male intersex, one intersex, one female intersex, and forty females. The major classifications are females and intersex types in the ratio of 1:1. If the genotype of the female was (XX Aa GG), and that of the intersex was (XY Aa GG), the resulting expected progeny would be four female to one intersex to two male intersex to one male. This fits the data obtained. The ratios of the intersex types vary somewhat, as was to be expected, due to the close balancing of sex factors and their reaction to environmental influences.

Discussion

It has been shown from the data collected over four generations of controlled breeding of spinach, that the inheritance

of sex is not a simple pattern, but is a complex of several factors located on more than one pair of chromosomes.

The assumption of an X-Y mechanism was necessary to fit a 1:1 ratio. The number of factors making up the sexual block of the X-Y chromosomes is not known, but it appeared to be more than a single factor.

The A-G gene linkage group and the YY type was based upon the genetic data of intersex plants segregating for males and females. The occurrence of a low crossover frequency was utilized to explain the intersex plants.

Further study, using the material which now appears to be known genotypically, should clarify some of the still-obscure patterns of intersex type segregation.

The various responses of the different genotypes to environmental effects need further study. This is a very complex problem, and, as there are two variables, it would be a considerable task to obtain conclusive results.

SUMMARY

Cytological Study

Spinacia oleracea L. has a chromosome number of twelve ($n = six$).

There are differences between lines concerning the number of nucleolar chromosomes, and in no line was there evidence of a heteromorphic chromosome pair. There were no chromosomal differences observed between male and intersex lines.

Tetraploid spinach was produced and studied, and the production of pollen was observed to be irregular.

Genetic Study

A scheme was suggested to fit the observed sex inheritance. It was primarily an X-Y system, plus two pairs of linked modifying genes, independent of the X-Y system, and having a very low crossover frequency. The existence of YY males and YY intersexes was assumed, based on genetical data and cytological observations.

Color Factor A, a single-gene type of inheritance, can be used as a marker gene for crosses between intersex plants where emasculation is an inhibiting factor.

Environment is a factor which affects the expression of certain classes of intersexes.

Sex expression affects the development of flower stalks, but the initiation of flower stalks is controlled by a separate factor or factors independent of sex expression.

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APPENDIX

OTHER GENETIC CHARACTERS OBSERVED IN S. OLERACEA L.

Besides sex expression, other characters which are genetical in nature were observed during the course of the investigation.

Color Factor A

This is a single-gene factor, for red color at the stem-root area. The area which will show the color varies from one-half inch to three inches, depending upon the size of the plant, and attains maximum intensity when the plants are in full flower. Red is dominant over colorless, with most commercial varieties being heterozygous for the factor. Data from the last three generations shown in tables in the Appendix indicate the nature of the inheritance of this gene. This character could be used as a marker gene in the crossing of intersex plants where emasculation is impossible in practice. By the use of a homozygous recessive intersex plant, a cross can be made with a homozygous dominant pollen parent plant, and when the progeny is grown, all red-stem (Color Factor A) plants will

be crossed individuals, and all white-stem plants will be the results of selfed seed.

Color Factor B

This factor was noted on the morphologically extreme male plant used in crossing. It is apparently a single-gene type inheritance, but this has not been conclusively shown. In this case, the red color persists very pale throughout the flower stalk, and if anthers are formed the anther wall becomes quite red. This factor, if present, will mask Color Factor A.

Horned-seed Factor

All of the plants bearing seed which were crossed with this morphologically extreme male expressed the horned-seed condition. This is in agreement with Nohara (1923), who showed that this horned-seed factor had a single-gene type of inheritance, and the horned seed was dominant over the round seed. From observations of horned seed, it was noted that the seed had from two to four spines, or horns, and that in some round-seeded types, small horns were observed on seed from some plants.

The Morphologically Extreme Type Male Plant

All males from the cross with this type male plant were of the same type, being morphologically extreme. This also appears to be a dominant condition, and is fully expressed only by male plants.

Savoyed Type Leaf and Dwarf Type Flower Stalk

Savoyed type leaf and dwarf type flower stalk conditions have been observed, but their mode of inheritance is not known.

Fasciation and Proliferation of Growing Points

One inbred line was observed for this character. The flower stalks all became fasciated, and, as the plant reached full maturity, the tips of the fasciated portions of the stem became a mass of growing points. Its mode of inheritance is unknown, but it appears to be a recessive character.

Response to Photoperiod

Spinach remains in the vegetative state until a certain length of photoperiod exists. The length of photoperiod required

to stimulate flower stalk development varies with different plants. Some of the foreign plant introductions of S. oleracea were observed to respond to a photoperiod of nine hours, while most commercial varieties require a photoperiod of from twelve to fourteen hours to initiate flower-stalk development. The sex of the plant modifies the rate of flower-stalk development, but apparently has little influence on floral initiation. It was observed that intersex and female plants will remain vegetative longer than male, if the populations observed were of the same genotype for photoperiodic response. However, it was also observed that females and intersex plants will form flower stalks ahead of male plants.

The explanation for this is the inherent response to photoperiod within the various plants. Another indication of this is that the intersex plants which were used in this experiment were usually selected early, and as a result were unconsciously selected for response to shorter photoperiods, as indicated by the early flower-stalk formation in the progenies grown in the early spring.

SEX REVERSAL

Description of Parent Plant of Progeny (XF-14-12-8-38)

A red-stemmed female plant grown in the greenhouse in the winter of 1951-52 produced a very few anthers (about ten to fifteen) near the lower portion of the main flower stalk, while the remainder of the plant was female. The main flower stalk reached a height of 3 feet, and was all female, when an apparent "sex reversal" took place within the last 5 inches of the main flower stalk. Within this 5-inch span, the flowers transformed from the pure female condition through a series of the monoecious complex and hermaphroditic flowers to a pure male condition, which terminated the flower stalk. Similar conditions existed to a lesser extent on the laterals.

The only seed which was set on this plant developed near the base of the main flower stalk, where the first anthers appeared, and a larger number of seeds was set from the terminals of the flower stalks where the later anthers appeared. The major portion of the flower stalk, which was pure female, failed to set seed, probably due to the lack of pollen.

While this was a manifestation of environment, it was not universal for all the female plants grown under this condition, but only appeared on a few plants. This indicates, again, that it is a combination of internal factors (genetic control) and external factors which determine this "sex reversal" in spinach.

PEDIGREE EXAMPLES

Planting Number One, Spring, 1952
(XF-14-11-15-1)

In the spring of 1950, an intersex plant was selected from a field planting of Long Standing Bloomsdale and Nobel. The plant selected was of the Long Standing Bloomsdale variety, with no control over the male parent; however, this plant was an intersex which was ten per cent female and ninety per cent male. This plant was designated as (XF). The seed from this plant was planted in the greenhouse in the winter of 1950-51. From the progeny of this planting an intersex was selfed. This plant was designated as (XF-14). The seed from this plant was planted in the field in the spring of 1951. A white-stemmed intersex plant was selfed from the progeny and was designated as (XF-14-11). The seed from this plant was planted in the greenhouse in the winter of 1951-52. From the progeny of this planting, a white-stemmed intersex plant was selfed. This plant was designated (XF-14-11-15). The seed from this plant was planted in the field in the spring of 1952, and the progeny

from this planting was designated (XF-14-11-15-1). The pedigree would then be listed as follows: (XF); (XF-14); (XF-14-11); (XF-14-11-15); (XF-14-11-15-1).

Planting Number Forty-seven, Spring, 1952
 [(XF-3-2) x (XF-3-2)-1-47]

(XF); (XF-3); (XF-3-2) x (XF-3-2); [(XF-3-2) x (XF-3-2)-1];
 [(XF-3-2) x (XF-3-2)-1-47].

For descriptions of the individuals, consult Tables VIII, IX, X, and XI in the Appendix, and for descriptions of the progenies of individuals, consult Tables XII, XIII, XIV, and XV in the Appendix.

TABLE VIII

PLANTING PLAN, WINTER, 1950-51
(all seed-bearing plants were of the Long Standing
Bloomsdale variety; male parent was unknown)

1. (XF) female	9. (XF) female
2. (XF) female	10. (XF) intersex
3. (XF) female	11. (XF) intersex
4. (XF) female	12. (XF) intersex
5. (XF) female intersex	13. (XF) male intersex
6. (XF) female intersex	14. (XF) male intersex
7. (XF) female intersex	15. (XF) female
8. (XF) female intersex	

TABLE IX
PLANTING PLAN, SPRING, 1951

1. (XF-3) intersex	7. (XF-11) intersex
2. (XF-3) intersex	8. (XF-12) intersex
3. (XF-6) intersex	9. (XF-14) intersex
4. (XF-7) intersex	10. (XF-14) intersex
5. (XF-9) intersex	11. (XF-14) intersex
6. (XF-11) intersex	12. (XF-14) intersex

TABLE X
PLANTING PLAN, WINTER, 1951-52

Pedigree No.	Sex	Stem Color
1. (XF-3-2)x(XF-3-2)	male x female	red x white
6. (XF-14-9)	intersex	red
7. (XF-14-11)	intersex	red
8. (XF-14-12)	intersex	red
12. (XF-14-9)	intersex	-
15. (XF-14-11)	intersex	white

TABLE XI
PLANTING PLAN, SPRING, 1952^a

Pedigree No.	Sex	Stem Color
1. (XF-14-11-15)	intersex	white
2. (XF-14-11-15)	intersex	white
3. (XF-14-11-15)	intersex	white
4. (XF-14-11-15)	intersex	white
5. (XF-14-11-15)	intersex	red
6. (XF-14-11-15)	intersex	white
7. (XF-14-11-15)	intersex	white
8. (XF-14-11-15)	intersex	white
9. (XF-14-11-15)	intersex	white
18. (XF-14-11-15)	intersex	white
20. (XF-14-11-7)	intersex	red
21. (XF-14-11-7)	intersex	red
22. (XF-14-11-7)	intersex	white
23. (XF-14-11-7)	intersex	red
24. (XF-14-11-7)	intersex	red
25. (XF-14-11-7)	intersex	red
26. (XF-14-11-7)	intersex	red

TABLE XI (Continued)

Pedigree No.	Sex	Stem Color
27. (XF-14-9-12)	intersex	white
28. (XF-14-9-12)	intersex	red
29. (XF-14-9-6)	intersex	red
30. (XF-14-9-6)	intersex	red
31. (XF-14-9-6)	intersex	red
32. (XF-14-9-6)	intersex	red
33. (XF-14-9-6)	intersex	red
36. (XF-14-12-8)	intersex	red
37. (XF-14-12-8)	intersex	red
38. (XF-14-12-8)	intersex	red
40. [(XF-3-2)x(XF-3-2)-1]	intersex	red
41. [(XF-3-2)x(XF-3-2)-1]	intersex	red
42. [(XF-3-2)x(XF-3-2)-1]	intersex	red
43. [(XF-3-2)x(XF-3-2)-1]	intersex	white
44. [(XF-3-2)x(XF-3-2)-1]	intersex	red
45. [(XF-3-2)x(XF-3-2)-1]	intersex	white

TABLE XI (Continued)

Pedigree No.	Sex	Stem Color
46. [(XF-3-2)x(XF-3-2)-1]	intersex	white
47. [(XF-3-2)x(XF-3-2)-1]	intersex	red
48. [(XF-3-2)x(XF-3-2)-1]	intersex	red
49. [(XF-3-2)x(XF-3-2)-1]	intersex	red
50. [(XF-3-2)x(XF-3-2)-1]	intersex	white
11. (XF-14-11-15) x (F.P.I.)	intersex x male	white x red
19. (XF-14-11-7) x (XF-14-11-15)	female x intersex	red x white
34. (XF-14-12-8) x (F.P.I.)	female x male	red x red
35. (XF-14-12-8) x (F.P.I.)	female x male	white x red
39. [XF-14-12-8] x [(XF-3-2)x(XF-3-2)-1]	female x intersex	red x red
52. (XF-14-11) ^b	intersex	red
53. (XF-14-9) ^c	intersex	red

TABLE XI (Continued)

Pedigree No.	Sex	Stem Color
54. (XF-14-11) ^d	intersex	white

^a Because of the environmental effects under greenhouse conditions, the intersex plants were all considered as intersex, and no attempt was made to further classify them. The color refers to the stem color.

^b Identical to number 7 in winter, 1951-52.

^c Identical to number 6 in winter, 1951-52.

^d Identical to number 15 in winter, 1951-52.

TABLE XII
DATA FROM WINTER, 1950-51, PLANTING

Pedigree No.	Male	Intersex	Female	Total
(XF-1)	17	-	9	26
(XF-2)	13	-	12	25
(XF-3)	17	2	12	28
(XF-4)	14	-	9	23
(XF-5)	9	-	14	23
(XF-6)	13	1	13	27
(XF-7)	10	1	12	23
(XF-8)	6	-	16	22
(XF-9)	10	2	8	20
(XF-10)	9	1	14	24
(XF-11)	12	2	12	26
(XF-13)	7	-	13	20
(XF-14)	6	4	4	14
(XF-15)	12	2	9	23
Total	161	16	169	346

TABLE XIII

DATA FROM SPRING, 1951, PLANTING

Pedigree No.	Male	Male Intersex	Intersex	Female Intersex	Female	Total	Stem Color	
							Red	White
(XF-3-2)	41	2	10	-	22	75	11	64
(XF-7-4)	12	2	17	15	17	63	19	44
(XF-9-5)	14	14	8	12	15	63	56	7
(XF-11-6)	4	3	15	2	14	38	37	1
(XF-11-7)	6	4	30	5	-	45	34	11
(XF-12-8)	10	7	22	11	16	66	66	-
(XF-14-9)	1	1	11	6	25	44	33	11
(XF-14-10)	3	19	4	4	2	32	20	12
(XF-14-11)	3	12	21	8	3	47	44	3
(XF-14-12)	24	14	41	3	-	82	11	71
Total	118	78	179	66	114			

TABLE XIV
DATA FROM WINTER, 1951-52, PLANTING*

Pedigree No.	Male	Inter- sex	Fe- male	Total	Stem Color	
					Red	White
(XF-14-9-6)	-	8	72	80	71	9
(XF-14-9-12)	-	4	18	22	20	2
(XF-14-11-7)	-	11	98	109	85	24
(XF-14-11-15)	-	6	63	69	3	66
(XF-14-12-8)	-	-	31	31	24	7
(XF-3-2)						
x	6	19	19	44	23	21
(XF-3-2)						

* The data on sex expression is not valid, as some of the plants scored as female later turned out to be intersex plants, producing a good set of seed.

TABLE XV
DATA FROM THE SPRING, 1952, PLANTING

Pedigree No.	Male	Nonfunc- tional Male Intersex	Male Intersex
(XF-14-11-15-1)	-	-	1
(XF-14-11-15-2)	-	-	10
(XF-14-11-15-3)	-	1	6
(XF-14-11-15-4)	-	1	1
(XF-14-11-15-5)	-	-	20
(XF-14-11-15-6)	-	-	-
(XF-14-11-15-7)	-		3
(XF-14-11-15-8)	-	-	3
(XF-14-11-15-9)	-	1	19
(XF-14-11-15-18)		-	14
(XF-14-11-7-20)		-	13
(XF-14-11-7-21)	-	12	47
(XF-14-11-7-22)	-	-	26
(XF-14-11-7-23)	-	8	44
(XF-14-11-7-24)	1	6	72

TABLE XV (Continued)

Intersex	Female Intersex	Female	Total	Stem Color	
				Red	White
45	3	-	49	-	49
68	-	-	78	-	78
33	1	-	41	1	40
55	1	-	58	1	57
56	2		78	60	18
110	2	-	112	-	112
73	4	-	80	3	77
53	5	-	61	-	61
36	1		57		57
36	-		50		50
42	-		55	55	
6	-	-	65	65	-
52		32	110	1	109
16	-	-	68	54	14
12	-	-	91	72	19

TABLE XV (Continued)

Pedigree No.	Male	Nonfunc- tional Male Intersex	Male Intersex
(XF-14-11-7-25)	-	-	43
(XF-14-11-7-26)	-	7	84
(XF-14-9-12-27)	-	-	13
(XF-14-9-12-28)	-	1	31
(XF-14-9-6-29)		3	58
(XF-14-9-6-30)	-	-	72
(XF-14-9-6-31)	-	4	34
(XF-14-9-6-32)	-	1	71
(XF-14-9-6-33)	-	-	14
(XF-14-12-8-36)		1	-
(XF-14-12-8-37)	-	1	6
(XF-14-12-8-38)	6	35	41
[(XF-3-2)x(XF-3-2)-1-40]	47	-	6
[(XF-3-2)x(XF-3-2)-1-41]	28	39	7

TABLE XV (Continued)

Intersex	Female Intersex	Female	Total	Stem Color	
				Red	White
40	-	-	83	61	22
21			112	84	28
36	1	-	50	-	50
13	-	-	45	32	13
13	-	-	74	56	18
23	-	-	95	77	18
23	4	1	66	49	17
3	-	-	75	75	-
55	12	-	81	61	20
8	15	21	45	31	14
17	24	13	61	46	15
-	-		82	61	21
3	-	20	76	54	22
-	1	21	96	74	21

TABLE XV (Continued)

Pedigree No.	Male	Nonfunc- tional Male Intersex	Male Intersex
[(XF-3-2)x(XF-3-2)-1-42]	83	8	-
[(XF-3-2)x(XF-3-2)-1-43]	41	19	14
[(XF-3-2)x(XF-3-2)-1-44]	15	16	20
[(XF-3-2)x(XF-3-2)-1-45]	10	38	14
[(XF-3-2)x(XF-3-2)-1-46]	17	22	28
[(XF-3-2)x(XF-3-2)-1-47]	-	9	23
[(XF-3-2)x(XF-3-2)-1-48]	9	16	31
[(XF-3-2)x(XF-3-2)-1-49]	3	11	21
[(XF-3-2)x(XF-3-2)-1-50]	35	27	3
(XF-14-11-15-11) x F.P.I. intersex x extreme male	39*	-	-
(XF-14-12-8-34) x F.P.I. female x extreme male	53*	-	-
(XF-14-12-8-35) x F.P.I. female x extreme male	31*		-
[XF-14-12-8-39] female x [(XF-3-2)x(XF-3-2)-1-40]	1	12	29

TABLE XV (Continued)

Intersex	Female Intersex	Female	Total	Stem Color	
				Red	White
-	-	24	115	86	29
4	2	21	101	4	97
-	2	18	71	54	17
7	-	21	90	3	87
4	1	24	96	-	96
19	1	19	71	52	19
1	-	25	82	58	24
7	2	19	63	49	14
-	-	19	84	1	83
23	16		78	78	
-	-	41	94	94	-
-	6	26	63	63	
1	1	41	85	85	

TABLE XV (Continued)

Pedigree No.	Male	Nonfunc- tional Male Intersex	Male Intersex
(XF-14-11-7-19) female x (XF-14-11-15-6)	-		-
(XF-14-11-7)	-	1	8
(XF-14-9-6)	-	-	11
(XF-14-11-15)	-	-	3

* Denotes extreme male.

TABLE XV (Continued)

Intersex	Female Intersex	Female	Total	Stem Color	
				Red	White
9	84	4	97	47	50
36	16	10	71	45	16
12	6	3	32	24	8
8	-	-	11	-	11

TABLE XVI

DATA FROM SPRING, 1950, TESTING SPACING AND DATE
OF PLANTING ON THE SEX EXPRESSION OF SPINACH

Planting	Date	Male	Intersex	Female	Total
<u>Long Standing Bloomsdale</u>					
1	4/22	46	10	35	91
2	4/29	141	33	137	311
3	5/6	152	27	148	327
4	5/13	142	24	146	312
5	5/20	117	11	93	221
<u>Spacing</u>					
Total		598	105	559	1,262
(1" - 2")		298	50	287	635
(4" - 6")		190	36	178	404
(8" - 10")		110	19	94	223

TABLE XVI (Continued)

Planting	Date	Male	Intersex	Female	Total
<u>Nobel</u>					
1	4/22	20	1	17	38
2	4/29	56	3	52	111
3	5/6	91	4	85	180
4	5/13	89	3	101	193
5	5/20	54	1	71	126
<u>Spacing</u>					
Total		310	12	326	648
(1" - 2")		129	5	143	277
(4" - 6")		98	2	114	214
(8" - 10")		83	6	69	157