Mercurialis (Gabe, 1939). In Mercurialis the YY plants produce very little pollen. Among the staminate progeny from selfing staminate plants, there was a ratio of 2 heterozygous (XY) for sex transmission to 1 homozygous (YY) for sex transmission.

6. Crosses of diploids with autotetraploids

Chromosome doubling in a dioecious species (one sex XX, the other XY) would be expected to produce two types of individuals: XXYY and XXXX. If the $\male$ is heterogametic, the cross of $4n\female$ x $2n\male$ (XXXX x XY) should produce XXX females and XXY males in about equal numbers, and $2n\female$ x $4n\male$ (XX x XXYY) should produce about $5\male : 1\female$. If the $\female$ were heterogametic, the same cross (now XXYY x XX) should produce 4 XXY: 1 XYY: 1 XXX or $5\female : 1\male$. Such tests in Spinacia oleracea (Janick, 1955) and in Melandrium album (Warmke and Blakeslee, 1939, Westergaard, 1940) showed that the $\male$ is heterogametic.

A similar test in Silene otites showed that the male is heterogametic (Warmke, 1942); although earlier tests by Correns (1928) and Sansome (1938) had suggested that the female is heterogametic.

If the heterogametic condition in the diploid is associated with a heteromorphic chromosome pair, preferential pairing in an XXYY individual might be expected and a high proportion of XY gametes. The staminate: pistillate ratio observed by Janick and Stevenson (1955a) from the cross of $2n(XX)\female$ x $4n(XXYY)\male$ varied from 2:1 to 5:1. A 5:1 ratio would indicate random pairing. The observed results suggest an excess of segregations of X chromosomes to one pole and Y's to the other.

7. Sex ratio among progeny of trisomics

In spinach, in crosses of diploid pistillate x trisomic staminate crosses, only the trisomic for chromosome 1 showed a trisomic ratio of $1\male : 2\female$ (Janick et al. 1959). No difference could be found in chromosome morphology associated with this chromosome pair (Ellis and Janick 1960).

Sex expression

Sex expression must be considered as a complex character under the control of many genes (Emerson 1924). One of the difficulties of analysis has been, as Allen (1940) pointed out, that maleness and femaleness have been thought of as different from other complex characters. For example, if size were considered in a manner similar to that in which sex has been considered, then every gene would be thought of as one for largeness or for smallness instead of thinking of each as contributing some component of the end result. Both the primary and secondary sex characters are gene controlled the same as other characters. The sex-comb, normally borne on the foreleg of the male Drosophila is absent in one mutant, double in another, and very large in another. Other mutants produce malformed eggs, others have rudimentary ovaries; defective ducts, spermathecae, or genitalia. The particular combination of factors affecting sex expression is determined at the time of fertilization but sex-differentiation and final sex-expression depend on the interaction of these genes with their environment, both internal and external.

In pigeons, the sexes cannot be distinguished until the birds are sexually mature. A scheme using sex-linked characters to permit early identification of the sexes was proposed by Gottfried and Hollander (1946).

In higher plants, the kind of gametophyte seems to be determined by which kind of structure, anther or ovary, is produced by the sporophyte. The primitive type of floral condition was probably the hermaphroditic one, in which the flowers were fully bisexual. There is a range of types from these to those fully unisexual with no obvious trace of the organs of the other sex. In most dioecious species, the flowers contain rudimentary organs of the other sex. The same is true of the unisexual flowers in monoecious species.

A few of the various combinations of types of sex expression on the same plant or on different plants and the terms applied to each are the following:

hermaphroditic - perfect flowers

monoecious - separate ♂ and ♀ flowers, but on the same plant

dioecious - separate ♂ and ♀ flowers, but on different plants

andromonoecious - perfect and staminate on the same plant

gynomonoecious - perfect and pistillate on the same plant

trimonoecious - the three types on one plant

androdioecious - two types of plants; perfect-flowered ones and staminate-flowered ones

gynodioecious - perfect-flowered plants and separate pistillate-flowered ones

Theories of sex determination

Quantitative or balance theory

Various grades of intersexuality without any change in the sex chromosomes were produced at will by crossing different strains from different geographical races of the gipsy moth (Lymantria dispar) (Goldschmidt 1911 to 1920, cf. Goldschmidt, 1931, 1955). These results were explained as the result of a balance between male and female determining genes of different potencies. Within a particular race, two doses of the gene determiners in the X produced the XX sex whereas only one dose produced the XY sex. As described by Goldschmidt (1934, 1938), crosses within each race gave normal sex ratios (the ♀ is XY, the ♂ XX), but crosses between races gave unexpected results. For example, a certain cross gave all intersexes; another only males. Also reciprocal crosses often behaved differently.

The intersexes were of two types, the female intersex which started as a ♀ (XY) and developed ♂ characters later, or the ♂ intersex which started as a ♂ (XX) and developed ♀ characters later; each series terminated by complete sex reversal. The degree or grade of intersexuality depended on the time in development at which the turning point toward the other sex was reached. The chromosome number remained the same in all parts. The following results are typical of the extensive experimental data from F_1, F_2, backcrosses, and reciprocals involving two or more races.

Cross 1: European ♀ (weak), x Japanese ♂ (strong) = F_1 normal ♂ , ♀ intersex.
Cross 2: The reciprocal cross: Japanese ♀ (strong) x European ♂ (weak) = F_1 normal ♀, normal ♂ .

The results were explainable if the X chromosome carried a set of M factors for maleness, and if a set of F factors for femaleness were carried outside of the X. The difference in behavior of reciprocals would be expected if the F factors were Y-linked or carried in the cytoplasm. In Lymantria they proved to be in the Y (Goldschmidt, 1920, 1955).

The male and female factors were assumed to have different "strengths" in different races, but similar "strengths" within a pure race. For example, to explain the above results, using the subscripts $\underline{w}$ and $\underline{s}$ to indicate relative strengths, weak or strong, cross 1 would be represented as:

weak ♀ x strong ♂   reciprocal (Cross 2): strong ♀ x weak ♂

$P_1 = F_w M_w \times M_s M_s$ $P_1 = F_s M_s \times M_w M_w$

$F_1 = M_w M_s = ♂$ $F_1 = M_s M_w = ♂$
and
$F_w M_s = ♀$ intersex $F_s M_w = ♀$

The balance between the female and the male factors of various strengths was assumed to determine the type of sex expression. Crosses between races with different "strength" alleles gave the intersexes. By assigning "strength" values it was possible to predict the behavior in crosses. The differences in strengths of the M factors behaved as alleles. By 1934, 13 alleles had been identified. They seemed to be strictly additive. Also eight different strengths of the ♀ factor in the Y chromosome had been identified. The strength of the $\underline{F}$ factor was not modified by being associated with an M of different strength through many hybrid generations. Also there was no indication of sex determiners in the autosomes from different sources (see Goldschmidt, 1955, p. 429).

His conclusions as to sex determination in Lymantria were:

1. Sex is determined by a quantitative balance between male and female factors; XY = ♀, XX = ♂ and F>M < MM, i. e. two doses of M are necessary to shift the balance to male.

2. The male factors behave as units, either a single gene or a group of closely linked ones.

3. The differences in "strength" were accounted for by supposed differences in "efficiency" of the sex-determining genes in the different races, i. e. differences in the rate at which these genes produce their sex-determining substances.

Winge (1937) advanced an hypothesis including autosomal determiners to explain Goldschmidt's results, but this does not fit the observed data *as well*.

The gene balance theory was applied also by Bridges (1925a) as an explanation for individuals with mixtures of female and male parts (intersexes) among the progeny of triploids in Drosophila melanogaster. Individuals with different chromosome numbers showed different grades of intersexuality. On the basis of further cytological and genetical studies, Bridges advanced the hypothesis that the X chromosome and the autosomes carry both male- and female-determining genes. That the balance for the X is female-determining is "shown by the fact that the addition of an X to a male group changes the individual into a female". The balance for the autosomes is male-determining, since the addition of a set of autosomes, as in 2X3A, causes the female to develop male characteristics and become an intersex. Since 2X2A is a normal female, the net female tendency of an X is greater than the net male tendency of a set of autosomes. By representing the net effectiveness of the female tendency genes in the X as 100 and the net male effectiveness of a set of autosomes as 80, a normal female has a ratio of 200:160 or a sex-index of 1.25:1 and a normal male of 100:160 or 0.63:1. These indexes and those for other X:A combinations are shown in Table 119.

Table 119. Relations of sex to chromosomes in <u>Drosophila</u> <u>melanogaster</u> (Bridges, 1939. Table 1, p. 40 from "Sex and Internal Secretions", 2nd Ed. Williams and Wilkins Co., Baltimore).

Sex-type			Formula	X = 100	A = 80	Sex-index (X/A)
Super	female	2n+X	3X+2A	300	160	1.88
Normal	female	4n	4X+4A	400	320	1.25
"	"	3n	3X+3A	300	240	1.25
"	"	2n	2X+2A	200	160	1.25
"	"	1n	1X+1A	100	80	1.25
Intersex		4n	3X+Y*+4A	300	320	0.94
"		3n	2X+Y*+3A	200	240	0.83
Normal	male	2n	X+Y*+2A	100	160	0.63
"	"	4n	2X+2Y*+4A	200	320	0.63
Super	male	3n	X+2Y*+3A	100	240	0.42

* The Y chromosomes were not listed by Bridges.

As shown in Table 119, Bridges' balance scheme applied to diploids as well as to the aneuploids of <u>Drosophila</u>, contrary to the statement by Goldschmidt (1955) that it applied only to aneuploids. Also, based on Bridges' scheme, the 4n, 3n and 1n types in which the number of X chromosomes and the number of sets of autosomes were the same, have the same index. This agrees with the observation that all were female with no "strictly sexual difference between them".

Subsequent experiments were set up to test the X and the autosomes for the presence of genes affecting sex expression. Tests of duplications for different sections of the X showed that all caused a shift in the female direction (Dobzhansky and Schultz 1934, Pipkin, 1940a). Hence there is no single sex-determining locus in the X. A deficiency of euchromatin shifted the expression in the male direction while a deficiency including mostly heterochromatin had no effect.

Sex expression was not affected by addition or loss of chromosome 4. Tests for the effects of duplications of portions of chromosomes 2 and 3 on sex expression have shown no marked shifts in sexual differentiation as a result of the change in dosage (Pipkin 1959, 1960). Translocations between chromosomes 2 and 4 and 3 and 4 were used in those studies. Hence the assumption of numerous male and female determiners in the autosomes, with a preponderance of ♂ factors has not been verified. The results suggest that hyperploidy for less than a full set of autosomes may not be effective.

In the silkworm, <u>Bombyx</u> <u>mori</u>, similar studies of sex expression in haploids, diploids and tetraploids with different doses of Z and W chromosomes, as summarized by Kihara (1953), gave very different results from those in <u>Drosophila</u>. Normally the male is ZZ and the female is ZW. In the absence of W, all individuals were males, regardless of the number of doses of autosomes or of Z chromosomes. Whenever W was present, the individual was female. No intersexes have been found and no W-linked characters. Experiments in which different fragments of the W were missing indicate that the factors for sex determination are confined to a certain section of the W chromosome (Tazima, 1943). A translocation which united that section with a portion of an autosome carrying a gene for egg color has been utilized for the visual separation of males and females in the egg stage (Tazima and associates, 1955).

In man (cf. Ford, 1960) and in mice, (Welshons and Russell, 1959 Russell, 1961) individuals with a Y are male; individuals with no Y, whether XX or XO, are female. In man the XO♀ is sterile.

In Melandrium (Lychnis), studies of sex expression in diploids, triploids and tetraploids with different doses of X and Y chromosomes show that a plant is male when one or more Y chromosomes are present and a female if it is absent (Warmke, 1946, Westergaard, 1940, 1948). The female potentialities showed some expression only when the ratio of Y:X reached 1:4. In Warmke's strains such plants had mostly hermaphroditic flowers, but in Westergaard's strains they were mostly males or only slightly hermaphroditic. The number of autosomal sets did not visibly affect sex expression. Here, the Y chromosome is larger than the X. Plants with fragment Y chromosomes (i. e. X Yf) have been studied for the effect of different segments on sex expression (Westergaard, 1946). As shown in Figure 55, segment IV in the X and in the Y are homologous. These ends pair at meiosis. The remainders of the two chromosomes are differential segments. The effects of two main fragment types were studied, Y^3 which had lost part of the arm carrying the homologous segment and Y^1 which had lost part of the other arm. Plants that had XY^1 had hermaphroditic flowers. Hence, the distal segment of that arm carries a gene or genes which suppress the formation of female sex organs. Plants that had XY^3 were male-sterile, but degeneration occurred after passing through normal meiosis. The distal segment of this arm must contain genes which control the last stages in development in the anthers after meiosis. Since XX plants are females, the middle portion of Y must control the initiation of anther development. Since these genes are present in a portion which is not homologous with any portion of the X, they are never separated by crossing over.

In spinach also, individuals with a Y chromosome are staminate, whether XY, XXY, or XXXY (Janick, 1955).

In Sphaerocarpos, certain fragments of the X chromosome changed sex completely with no intersexual types (Knapp and Hoffmann, 1939). Hence the smallest one with this effect must have had all the necessary sex differentiating genes.

FIG. 55. Schematic drawing of the sex chromosomes of Melandrium. I, II, III: the differential segment of Y, IV: the homologous segments, V: the differential segment of X. Segment I has the female suppressor action, II initiates anther development, and III controls the late stages in anther development (Re-drawn from Westergaard, 1948).

Bridges (1925a) applied the genic balance hypothesis to the sex types in a dioecious moss obtained by the Marchals and Wettstein. By assigning different values to the autosome set and to the X and X' chromosomes which would account for the difference between ♂ and female; the sex expression of other polyploid types could be accounted for.

He also pointed out that in monoecious or hermaphroditic forms the haploid and higher polyploid forms show the same sex expression. On the genic balance hypothesis this is expected, since they all have the same ratio between chromosomes. Later evidence shows this is not true for all characters. Bridges also suggested that in dioecious species different 2n+1 types might be expected to show different sex expressions. Ono (1935) confirmed this prediction in Rumex acetosa. For example, one 3X, 3A+1 was a female, another 3X, 3A+1 an intersex.

Correns (1928) proposed that all angiosperms have potencies for femaleness in a gene or gene-complex G; for maleness in a gene or gene-complex A; and have another complex influencing the time and order of appearance of male and female organs. It
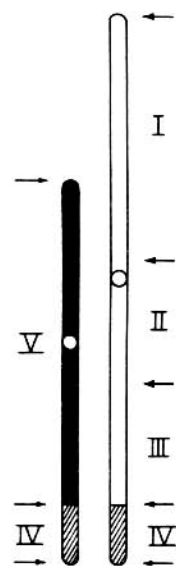
appears to be little more than a re-statement of the balance theory in a slightly different form.

Westergaard's proposed scheme to account for the evolution of dioecism will be presented later (p. 293).

The sex of haploids

In Hymenoptera and certain other genera, fertilized eggs develop into females while unfertilized eggs develop into males. In other words, an individual with n chromosomes is a ♂ , one with 2n is a ♀. In Drosophila both are females, since the gene balance is similar. An explanation for the difference in behavior in Hymenoptera was furnished by studies of sex determination in Habrobracon, a small wasp parasitic on larvae of the mealmoth, Ephestia. Cytological investigation (Torvik-Greb, 1935) showed that the female has 20 chromosomes while the male has 10.

In Habrobracon the cross of recessive orange-eyed ♀ x wild black-eyed ♂ produced black-eyed biparental ♀ and orange-eyed uniparental sons, termed "sex-linkoid" inheritance. However, in other crosses of the same type within related stocks, the above classes of offspring were found, but in addition black-eyed biparental males with 20 chromosomes. Whiting (1933) advanced the hypothesis that the females are heterozygous for a differential pair of factors or complexes affecting sex-expression $X^a X^b$. The uniparental males are $X^a A$(A = autosomes) or $X^b A$, while the biparental males are $X^a X^a AA$ or $X^b X^b AA$; thus holding the same genic balance as in the haploid males. Male mosaics from binucleate eggs showed femaleness in the zone between the two types, as might be expected if males are of two types, and if females carry the two factors or complexes (Whiting, 1935). All haploids are males, but not all males are haploids. Biparental males were missing in outcrosses, and occurred with increasing frequency as inbreeding progressed (Bostian, 1934).

Whiting (1943) reported 9 different alleles of X^a and X^b. The X was thought of as having a differential segment. An individual with N chromosomes is a ♂ but NN is also ♂. The female is N'N, not 2N.

In the Ichneumon Pteromalus puparum, a similar explanation is probable (Dozorceva, 1936). The diploid ♀ has a heteromorphic pair of chromosomes -- 4II + JV while the males are of two types: 4 + J and 4 + V.

It seems probable therefore that genic balance plus a series of alleles is also applicable to sex determination in other forms where haploid individuals occur regularly.

Sex-limited characters

In Lebistes reticulatus an aquarium fish, the ♂ is XY but both the X and Y may carry genes for male coloration. These are also sex-limited only being expressed in the male, whether on the X or the Y. Characters whose genes are carried on the Y are transmitted directly from father to son. Such genes carried on the X of a male are not expressed in the F_1 females, but the characters reappear in one-half of the F_2 males.

Certain genes for color may not be sex-limited. For example, the two characters gold and red spotted in Platypoecilis maculatus were reported by Fraser and Gordon (1929) to be sex-linked but not sex-limited. An interesting result in their experiment was the occasional exception in which characters that had been Y-linked became X-linked,

presumably as a result of crossing over between the X and Y. The two characters used were Red and Spotted (R and Sp) vs. gold (non-red (r) and non-spotted (sp)). The results obtained from heterozygous ♀, carrying R and Sp on the X and r sp on the Y, backcrossed to gold males, together with their interpretation in terms of X and Y chromosomes are as follows (the Y is represented as being structurally different from the X for purposes of illustration):

$$
\begin{array}{c}
R \\
Sp
\end{array}
\Bigg|\Bigg|
\begin{array}{cc}
r & r \\
sp & sp
\end{array}
\Bigg|\Bigg|
\;x\;
=
\begin{array}{cc}
r & r \\
sp & sp
\end{array}
\Bigg|\Bigg|
\;+\;
\begin{array}{cc}
r & R \\
sp & Sp
\end{array}
\Bigg|\Bigg|
\;+\;
\begin{array}{cc}
r & r \\
sp & sp
\end{array}
\Bigg|\Bigg|
\begin{array}{c}
R \\
Sp
\end{array}
$$

Red spotted x gold ♂ = 43 gold ♀ + 41 red spotted ♂ + 1 red spotted
    F₁ ♀   ♀ exception

The exceptional Red Spotted ♀ when crossed with a gold ♂ produced: 15 red spotted ♀ 17 gold ♂ and another exceptional red spotted ♂. . The progeny from the original red spotted exceptional ♀ are expected if R and Sp had crossed over from the X to the Y chromosome. The red spotted ♂ exception that again occurred, presumably represented a crossover of R and Sp from the Y back to the X.

This means that the portions of the X and Y which can be transferred are closely but not completely linked with the portion that carries the factors for sex determination. If a cytological difference between the X and Y is found later, and each is invariably associated with sex, then the sex factors must be closely or completely linked with this cytological difference, otherwise crossing-over would transfer the cytological marker to the other sex (Morgan, 1926a).

Winge (1934) also found two races in Lebistes in which the XX females showed male-tendencies. A cross between these resulted in a few XX individuals which were phenotypically and functionally males, and showed characters obtained from the mother in one X and from the father in the other X. These, when crossed with normal ♀, produced all females. The original XX♂ mated with these females produced 164♀ and no ♂. The original XX♂ when again mated with these daughters produced one XX♂ which when crossed to XX sisters produced about 1♂ :1♀. Here a homozygous XX line was established in which sex-determination was taken over by a pair of autosomes in one of which the male-tendency genes had become associated (i. e. brought together in one member of the pair). He was also able to select in XY males until he had XY females, which when mated to XY males produced 28♀:81♂ . Among the males 22 were YY.

An attempt to accomplish a similar result in Aplocheilus by accumulation of sex-tendency genes in an autosome was unsuccessful (Aida, 1936).

Sciara

Sex-determination in Sciara coprophila, a dipteran fungus-gnat, involves some interesting departures as to chromosome behavior and inheritance. An outline of certain features in species producing unisexual progenies, based on summaries by Metz (1938) and Bridges (1939) is shown in the diagram in Figure 56.

It will be noted that there are three types of zygotes, all of which are 3X. One of these (type 1) has one chromosome designated X' which differs by carrying a gene or genes which so change the germinal cytoplasm that (X) eggs developed in this X'XX zygote behave differently from the (X) eggs developed in an XXX zygote (type 2).