

CHAPTER 10 SEX DETERMINATION

Gametic union in a life cycle initiates a sequence of events which furnish the opportunity for genetic recombination. As Allen (1935) has pointed out, gametic union is not sexual reproduction. A life cycle in which gametic union does not occur produces individuals that are relatively uniform, although somatic crossing over may uncover recessives carried in heterozygous condition. Somatic crossing over has been demonstrated in *Drosophila melanogaster* by numerous experiments (Stern 1936). A process similar to somatic crossing over occurs in certain fungi, along with a form of somatic reduction. This has been referred to as parasexuality. These two processes, mitotic recombination and haploidization have been used to determine linkage groups and linear order of genes in *Aspergillus nidulans* (Pontecorvo and Kafer 1958, and Kafer 1958) and by others in several other species.

Sex factors in the bacterium, *Escherichia coli* "alternate between a state in which they are attached to the chromosome and a state in which they multiply autonomously and are independently transmissible" (cf. Adelberg 1960).

Genetic recombination has been demonstrated in bacteriophages (viruses) which reproduce in bacteria, suggesting some mechanism of exchange leading to segregation (cf. Adams 1959).

In higher organisms sex-expression is found in the sporophytic generation. In higher plants the microspores and the megaspores are produced in special organs, the anthers and the ovaries respectively in the sporophyte. The spores in turn undergo nuclear divisions, or nuclear and cell divisions to form the gametophytes. One of the cells of the ♀ gametophyte functions as an egg cell, and the generative cell of the ♂ gametophyte divides to form two sperms (Figs. 2 and 3). In higher animals, the gametophyte generation is reduced practically to zero, since the gametes arise from the products of meiosis without further divisions. Only the major points concerning reproduction following gametic union and the cytogenetic basis of sex determination will be considered here. For more extensive reviews consult: Bridges (1932, 1939), Crew (1925, 1954), Wilson (1928), Goldschmidt (1955), Makino (1951), White (1954, Chaps. 15-17), and Gowen (1961) for animals; Allen (1940), Westergaard (1958) and Gowen (1961) for Angiosperms; and Loehwing (1938) and Bonner and Galston (1952, Chapt. 17) for the physiological aspects in Angiosperms.

In animals, McClung (1902), the first to suggest that the accessory chromosome in the male exerted a special influence on sex, emphasized the parallel between the two equal classes of sperm and the 1:1 ratio of males and females. Cytological findings were reported by Stevens (1905) and subsequently by many others. The male was always the heterogametic sex. The first case of sex-linked inheritance was found in the currant moth, *Abraxas grossulariata* (Doncaster and Raynor, 1906), but the female appeared to be the heterogametic sex. The same was true for the linkage of sex with black vs. barred pattern of the Plymouth Rock fowl (Spillman, 1909); and for linkage with one

character in canaries (Durham and Marryat, 1908). Sex linkage in Drosophila with the male as the heterogametic sex was reported first by Morgan (1910). Miss Stevens observed the sex chromosomes in Drosophila melanogaster in 1912 using the aceto carmine smear technique, the ♂ being XY. Thus there appeared to be a discrepancy between the prediction based on cytology and the observed genetic linkages in certain species. The fact that the female could be the heterogametic sex cytologically was not reported until 1913 by Seiler for the Lepidopteran Phragmatobia fuliginosa. A convincing demonstration of the relation between the behavior of a particular chromosome, the X, and the inheritance of a group of characters was reported by Bridges (1916) for Drosophila.

In plants, the first cytological work on dioecious forms did not show any visible chromosome differences. The first case of a chromosome difference between sexes in plants was found by Allen (1917) in the Liverwort, Sphaerocarpos donnellii, where the female gametophyte was (7+X) and the ♂ was (7+Y). The first cases in angiosperms were found by Santos (1923) in the dioecious Elodea gigantea (water weed), by Kihara and Ono (1923) in Rumex acetosa, Blackburn (1923) in Melandrium, and Winge (1923) in Melandrium, Humulus and Vallisneria. In Elodea the four microspores from a given sporocyte remain together, and two were reported to be markedly larger.

Sex chromosomes in animals

Many variations in sex chromosome constitution and behavior have been found in insects and in various other groups of animals, see White, (1954) for an extensive account.

The most common types in animals are the following:

1. Male heterogametic.

- a. Protenor type: ♀ XX, ♂ X (an unpaired X), found in most Orthopterans (these include cockroaches, praying mantis, walking sticks, grasshoppers, and crickets); many bugs, beetles, spiders, myriapods, and nematodes.
- b. Lygaeus type: ♀ XX, ♂ XY, found in mammals, common in Diptera (Drosophila as an example), frequent in bugs and beetles. Man falls in this group.

2. Female heterogametic.

- a. ♀ XO or XY, ♂ XX (commonly referred to as ZW♀, ZZ ♂), found in Lepidoptera and Trichoptera in the insects; birds, and in certain fishes. The X may or may not have a mate in the female.

In some species the X, in others the Y chromosome may be multipartite (a compound X or Y).

In the wild Mexican platyfish, the male is heterogametic, but in the domestic race of the same species the female is heterogametic (Gordon 1947). Crosses between them showed that the Y and Z are homologous (Gordon 1946).

Sex chromosomes in plants

A list of the dioecious species of plants in which sex chromosomes had been reported was published by Allen (1940). Westergaard (1958) lists the species in which the presence of heteromorphic sex chromosomes has been well established; and those for which more evidence is needed. The presence of a heteromorphic pair in one sex must

be supplemented by observations on the other sex. The types of sex chromosome constitutions reported in plants include the following:

1. Male heterogametic - ♀, XX; ♂, XY
Most of the dioecious Angiosperms fall in this group. Well established examples are: Humulus lupulus (hop), Rumex angiocarpus, Melandrium album, and M. rubrum. Less well established examples are several species of Populus (Poplar), Salix (willow), Smilax, and Cannabis (hemp).
2. Male heterogametic, but XO; ♀, XX; in Vallisneria spiralis (Winge, 1923), also reported in Dioscorea sinuata (Yam), but more evidence is needed.
3. Male heterogametic, but with one extra chromosome, reported in Phoradendron flavescens (American mistletoe), and P. villosum, but more evidence is needed.
4. Female heterogametic. ♀, XY; ♂, XX, observed in 6X Fragaria elatior (Kihara, 1930). The breeding behavior indicates the same for other polyploid European species of Fragarias.
5. Compound chromosomes. In Rumex acetosa (garden sorrel) and in Humulus japonicus, in most instances there is a chain of 3 chromosomes in the heterogametic sex, arranged in meiosis as $Y_1 X Y_2$ with the X oriented to pass to one pole and Y_1 and Y_2 to the other pole (Jacobsen, 1957). In certain races there is a chain of five chromosomes in the ♂, probably the result of an interchange between one of the Y chromosomes and an autosome. In Humulus lupulus (the common hop) in certain races there is an unequal chromosome pair in the ♂ (Jacobsen, 1957). In one race, var. corduifolius, there is a chain of four chromosomes. Sinoto (1929a) gives its formula as: ♀ $2X_1 + 2X_2$; ♂ $X_1Y_1X_2Y_2$. Darlington (1937) described it as the result of an interchange between one of the sex chromosomes and an autosome. One sex is then a structural hybrid. The formula and the order of the chromosomes might be $A_1^{Y_1} - A_1 - Y_1^{A_1}Y_1$ with $A_1 + Y_1$ in one type of viable spore and the interchange chromosomes $A_1^{Y_1} + Y_1^{A_1}$ in the other. The female would have only bivalents, whereas the male would have an association of four chromosomes. For H. lupulus and H. japonicus, Winge (1923) had reported that the ♀ had $18 + X + X$ and the ♂ $18 + X + Y$, the Y smaller than the autosomes.

The mere presence of a pair of heterochromosomes is not sufficient evidence that they are sex-chromosomes, as Jensen (1940, 1942) and Westergaard (1958) have pointed out. However, Jensen denied their existence even in those that are well-established. Heterochromosomes may not always be associated with a difference in sex expression. For example, heterochromosomes were first found in plants in Galtonia candicans, an hermaphroditic species. Another hermaphroditic species, Rumex pallidus, (white dock), has a 3-fold complex of chromosomes of the kind designated as sex chromosomes in R. acetosa.

Allen (1940) also listed the dioecious species which had been examined without finding sex chromosomes. Notable examples are: Spinacia oleracea, Ribes alpinum, Vitis cinerea, V. rupestris, V. vinifera, Carica papaya, Asparagus officinalis, and Bryonia dioica. Others have been listed by Westergaard (1958). In Spinacia, the sex determining "factor pair" is on chromosome 1. The male is heterogametic. In some stocks this pair is heteromorphic but the heteromorphism shows no relation to sex determination (Janick and Bose, 1960).

General cytology

In general, the sex chromosomes may be larger than any of the other chromosomes or they may be smaller. In many forms they are largely heterochromatic. They are associated with the nucleolus in Drosophila and man and also in the dioecious liverworts. In Drosophila, most of the Y and a large portion of the X are heterochromatic. The heterochromatic portions are represented by only a few bands in the salivary gland chromosomes. The point of association of the X with the nucleolus in Drosophila melanogaster is between the 20B12 and 20C12 doublets a short distance from the nearly terminal centromere in Bridges' map (Kaufmann 1938).

In man the nuclei of somatic cells of normal females are characterized by one small chromatin body (often two of equal size) usually adherent to the nuclear membrane (Barr 1959). In whole mounts of thin membranes it can be identified in virtually every nucleus. In males this body is missing in most cells, but up to 10% of the nuclei in sections may have a small chromatin mass, rarely as large as in females. Observations on XXY, XXX, and XXXY individuals indicate that the number of chromatin bodies often is one less than the number of X chromosomes (see summaries by Gowen, 1961 and Lennox, 1961). The reason for this is not obvious.

Heteropycnosis in the nuclei of somatic cells as a means of diagnosing sex was demonstrated in the spruce budworm, Archips (Cacoecia) fumiferana, by Smith (1945). The heteropycnotic bodies, restricted to the nuclei of females, presumably were or were a part of the sex chromosomes.

Methods of determining the heterogametic sex

Four ways to determine which sex is heterogametic were listed by Correns (1928), two additional ones by Westergaard (1958) and one by Janick, namely:

1. Cytological identification of a heteromorphic pair of sex chromosomes, as discussed above.
2. Genetic linkage showing criss-cross or Y-linked inheritance.
3. Crosses between dioecious and monoecious species.
4. Competition tests in pollinations.
5. Self-pollination of bisexual flowers on normally unisexual plants.
6. Crosses of diploids with autotetraploids.
7. Sex ratio among progeny of trisomics.

1. *Cytological identification has been discussed earlier. The others will be discussed in some detail.*

2. *Genetic linkage, showing criss-cross or Y-linked inheritance.*

The few cases of sex linkage which have been reported in plants appear to behave similarly to those in animals.

a. The first one (Baur, 1912, Shull, 1914) was in Lychnis alba. The cross of broad-

leaved ♀ (normal) x narrow-leaved ♂ produced in F_1 all broad-leaved males and females, and in F_2 : the females were all broad-leaved; part of the males were broad, part narrow-leaved. Further breeding behavior also indicated sex linkage, this gene pair being located in the X with no allele on the Y. The few hermaphroditic types which appeared were in certain cases mutated males, still with XY; in others mutated females still with XX chromosomes.

- b. In F_2 of crosses between Lychnis dioica (red campion) and L. alba, (evening-Lychnis) Winge (1927, 1931, 1931a) found several characters segregating. One was a recessive aurea. Heterozygous green ♀ x aurea ♂ = green ♀, green ♂, and aurea ♂; but no aurea ♀. Homozygous aurea appeared to be lethal in the ♀ unless a dominant inhibitor A was present which caused them to be green. Another was a Y-linked inhibitor of variegation, so that only the females were variegated. The recessive, "abnormal plant", was borne on the X or Y or both. Its effect was inhibited by autosomal genes, as well as by aurea. In certain females, there was an X-linked lethal. It prevented the appearance of homozygous ♀ progeny. In hemp, Imai (1938) found two different mutants as males following X-raying of pollen. In Carica papaya, Hofmeyr (1938, 1939) reported linkage between flower color (Y = yellow, y = white), stem color (P = purple, p = non-purple) and the sex determining factors, M_1M_2m (see page 285); the 3-point linkage map being m 23.7 Y 17.3 P. In Rumex acetosa, Löve (1943) reported a Y-linked gene for asynapsis, with no dominant normal allele on the X. The cross of normal ♀ x asynaptic ♂ (almost completely pollen sterile) produced normal females and highly sterile males. When these ♀ offspring were crossed with asynaptic males the male progeny were asynaptic, the females normal. When the same ♀ offspring were crossed with normal males, the progeny, both ♂ and ♀, were normal. The ♀ trees of Broussonetia papyrifera, the paper mulberry, were reported to be less hardy than ♂ trees (Andrews 1940). In the north there are only ♂ trees.

The writer has been unable to find any published comparisons of crossing over in ♂ and ♀ of dioecious plants. Higher crossing over has been observed in microsporogenesis than in megasporogenesis of the same plant in maize (monoecious) (Rhoades, 1941a, Burnham 1949, and Clark 1956) and in Primula (hermaphroditic) (de Winton and Haldane, 1935).

3. Crosses between dioecious and monoecious species

In Bryonia, Correns (1907, 1928) reported that from B. dioica (dioecious) ♀ x ♂ there was a ratio of about $1♀ : 1♂$; from B. dioica ♀ x B. alba (monoecious) ♂ the offspring were all ♀; whereas from B. alba (monoecious) ♀ x B. dioica ♂ there was a ratio of $1♀:1♂$. Therefore, the ♂ of B. dioica is heterogametic. Dioecism seemed to be almost completely dominant in these crosses. The hybrids were sterile. These crosses have been repeated by Heilbronn and Basarman (1942) and Heilbronn (1953), and some backcrosses were obtained. The results were similar to those obtained by Correns. The crosses between another dioecious species B. multiflora and B. dioica ♂ gave a 1:1 ratio of ♂ and ♀; but the reciprocal cross gave only monoecious plants.

Crosses between dioecious Acnida species and two types of monoecious Amaranthus (pigweed) species have been reported by Murray (1940). Crosses with the "first type" monoecious species, in which the first flower of each cluster was ♂, the others ♀; gave results which indicated that the male in the Acnida species is heterogametic. Certain strains of Acnida tamariscina carried a gene which was expressed only in intergeneric crosses with Amaranthus species of the first type and produced non-flowering or neuter plants. Crosses of dioecious species of Acnida with Am. spinosus, the "second type" monoecious species in which the staminate flowers were in terminal

clusters, produced mostly staminate plants. This may be explained by assuming that in this intergeneric cross, genes from the monoecious parent have blocked development of the ♀ organs (a dominant effect), whereas in the crosses with type 1 monoecious species there was no such effect; i. e. the genes from the monoecious parent acted as recessives.

In Spain, there are monoecious and dioecious varieties of Ecballium elaterium. The cross of dioecious ♀ x monoecious ♂ gave all monoecious plants. The cross of monoecious ♀ x dioecious ♂ gave a ratio of about 1 ♂ : 1 dioecious (Galan, 1951, 1953). The results were explained by assuming the monoecious species to be $X^m X^m$ and the dioecious ♂ XY. Then $X^m X$ is monoecious but $X^m Y$ is a male. The X and Y refer to a sex controlling gene pair or group of genes.

In the Amaranthus and Acnida crosses:

$XX \times X^m X^m$ (type 1), $X^m X$ was a ♀;

in $X^m X^m$ (type 1) x XY, $X^m X$ was ♀ and $X^m Y$ was ♂; whereas in

$XX \times X^m X^m$ (type 2), $X^m X$ was a ♂, and in

$X^m X^m$ (type 2) x XY, $X^m X$ and $X^m Y$ were ♂.

4. Competition experiments, comparison of the sex ratios in progeny from sparse vs. excess pollen.

Correns (1907, 1917) found in Melandrium that plants pollinated with an excess of pollen gave an excess of ♀ progeny, whereas the ratio was about 1:1 with sparse pollen; and concluded that the ♂ was heterogametic. Similar experiments in Fragaria did not change the sex ratio and led him to conclude that the male was homogametic.

In Cannabis sativa (hemp), Riede (1925) found that sparse pollination increased the proportion of males.

5. Self-pollination of plants that are normally unisexual

In Asparagus (n = 10) Rick and Hanna (1943) found a few seed on ♂ plants. When these were grown, they produced a ratio of 3♂ : 1♀. Although phenotypically no different, one-third of these males were homozygous for male transmission, i. e. produced all males in crosses with females. This could have certain advantages commercially since ♂ plants are more vigorous and have a larger number of stalks although somewhat more slender; resulting in an increase in yield of about 25% (Robbins and Jones, 1925). The observed ratios can be explained by assuming the ♂ has one chromosome pair with a differential genic constitution. Although no sex chromosomes have been observed, an $XY\sigma$, when selfed should produce: $XX + 2XY + YY$ or a ratio of 1♀ : 3♂. From $XX\sigma \times YY\sigma$, all offspring should be XY and ♂. A pair of genes with maleness dominant also explains the results. Certain varieties or strains produce occasional hermaphroditic flowers, others do not. Snee (1953) reported the establishment by selection of an hermaphroditic strain which segregated hermaphroditic, female, and male plants.

McPhee (1925) obtained in Cannabis sativa (hemp) a ratio of 3♂ : 1♀ from selfing male plants which had a few female flowers. Sinoto (1929) found a bimodal curve for size of pollen. A ratio of 3♂ : 1♀ from selfs of males has been observed also in Spinacia oleracea (Janick and Stevenson, 1954), Thalictrum (Kuhn 1939) and in

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 σ^7 is heterogametic, the cross of $4n^{\circ} \times 2n^{\sigma}$ (XXXX \times XY) should produce XXX females and XXY males in about equal numbers, and $2n^{\circ} \times 4n^{\sigma}$ (XX \times XXYY) should produce about $5\sigma^7:1\phi$. If the ϕ were heterogametic, the same cross (now XXYY \times XX) should produce 4 XXY: 1 XYY:1 XXX or $5\phi:1\sigma^7$. Such tests in Spinacia oleracea (Janick, 1955) and in Melandrium album (Warmke and Blakeslee, 1939, Westergaard, 1940) showed that the σ^7 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 $2n(\text{XX})^{\phi} \times 4n(\text{XXYY})^{\sigma}$ 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 \times trisomic staminate crosses, only the trisomic for chromosome 1 showed a trisomic ratio of $1\sigma^7:2\phi$ (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).