

Cytoplasmic male sterile lines in maize have been established also from the iojap (ij) stock (Rhoades, 1943, 1950a). Lines homozygous for iojap showed male sterility in occasional plants, and in crosses of $\underline{ij} \underline{ij}^{\circ}$ with normal σ° , an occasional $\underline{Ij} \underline{Ij}$ F_1 plant was male sterile. By crossing again with normal, a male sterile plant that was $\underline{Ij} \underline{Ij}$, normal green and not carrying \underline{ij} , was produced. This is similar to the procedure by which a chlorophyll defective character showing cytoplasmic inheritance was established from ij (Rhoades, 1943). Ten different sources of cytoplasmic male sterility were listed by Jones (1955). The Texas source of cytoplasmic male sterility (T), reported by Rogers and Edwardson (1952), requires two major genes plus modifiers to restore complete fertility. These major genes have been designated \underline{Rf}_1 and \underline{Rf}_2 (Duvick 1956). Their action is similar to that of the \underline{Ms} gene and \underline{rf} to that of \underline{ms} described above for flax. One restorer gene was linked with two 1-3 interchanges (Linden 1956). Subsequent tests have shown that \underline{Rf}_1 is located in chromosome 3 between \underline{d}_1 and \underline{ts}_4 (Blickenstaff et al. 1958, Duvick et al. 1961). Most inbreds in the United States are $\underline{rf}_1 \underline{rf}_1 \underline{Rf}_2 \underline{Rf}_2$. There are different degrees of restoration. Varieties and inbreds vary widely in their ability to restore fertility. (Jones 1950, 1955, Newlin, 1955, Edwardson, 1955, Briggie 1957, Gabelman, 1949, 1952).

Jones and Everett (1949), Jones and Mangelsdorf (1951) and Rogers and Edwardson (1952) have outlined methods by which male sterility might be used to replace the usual detasseling needed to produce double cross hybrid seed. According to one plan, applied as an example to the double cross (AxB)x(CxD), only the inbred D or both C and D would carry fertility restoring factors. The AxB single cross used as the female parent is produced by crossing male-sterile A with B. Since B is free of restoring genes, this avoids detasseling of the single cross (AxB), as well as male-sterile A. A pollen fertile inbred A must be maintained as a pollen source to produce male-sterile inbred A (the male-sterile equivalent of A produced by successive backcrosses to pollen fertile A as the male parent). The method has been used successfully in the production of hybrid seed corn; and a larger and larger proportion of the hybrid corn sold is being produced in this manner.

The method has been successful for hybrid sorghum (Stephens and Holland, 1954, Quinby and Martin, 1954); and also for onions (cf. Jones and Clarke, 1947).

Sex ratios

In general, in dioecious plants, females are more numerous than males. In the hop, Humulus lupulus L., Holubinsky (1939) reported the percentages of males from 3 different females, each pollinated by several different males or a mixture of pollen as: 1%, 7.4%, and 23.9%. Correns reported from 9.8% to 22.8% of males. In Humulus japonicus, Kihara and Hirayoshi (1932) reported an excess of females following heavy application of pollen. Counts reported by Winge (1923) showed an excess of females in both species.

In Lychnis dioica, L. alba and Rumex acetosa, Correns (1928) found a larger proportion of males following scanty pollination, even approaching 50%. Aging the pollen or treatment of the dry pollen with alcohol vapor also increased the proportion of males. Differences in age of ovules had no effect. Tischler (1925) found an increased proportion of males from Lychnis pollen subjected to alcohol vapor at the time of germination. In all these cases, the pollen was of two kinds. The various treatments may have acted to change their competitive ability. Sex ratios (sex expression) may be modified also by the growing conditions (see latter part of this discussion).

In asparagus, the sex ratios resulting from the use of different staminate plants on the same series of pistillate parents were mostly 1:1 but an occasional cross produced a significant excess of males (Currence, 1954).

In *Cannabis sativa* (hemp), a wide range of types was found from strongly male habit with many female and few male flowers, through weak female and monoecious types and all transitions up to weak σ^7 and strong ♀ (Grischko, 1937, from a review by Sengbusch, 1942). The range of types occurred in both genotypic males and in genotypic females. Sengbusch proposed the hypothesis of a series of alleles of sex factors (F) of different strengths. An XX or XY combination might produce the same sex phenotype depending on the grade of each F factor carried by the X.

The results suggest the possibility of breeding a hemp in which the sex ratio is shifted in favor of the females, one in which males ripen at the same time as the females, or a monoecious type (Hoffmann, 1938, 1941).

In animals in which one sex is heterogametic the primary sex ratio (immediately after fertilization) should be 1:1 unless there is differential fertilization. The ratio at birth may deviate considerably from 1:1 due to differences in viability of the two sexes. The sex ratio at birth may not be strictly 1:1 as shown by a table given by Crew 1925 for the number of males per 100 females:

man	103-107
horse	98.3
dog	118.5
cattle	107.3
sheep	97.7
pig	111.8
rabbit	104.6
mice	100-118
fowl	93.4-94.7
pigeon	115.0

Differential motility between X- and Y-bearing sperms is one possible reason for unequal primary sex ratios.

In the fowl the primary sex ratio can be determined if every egg is fertile and all hatch. In a series of such trials there were large differences between families (Hays, 1945).

In the albino rat, King (1918) selected for 15 generations in two lines, one for a preponderance of ♀ , the other of σ^7 ; with the result in one of 83 σ^7 per 100 ♀ and in the other of 125:100. For rats and man, the sex ratio varies with the season of the year, the season with the greatest birth rate having the lowest proportion of males.

In man, older mothers produce fewer male offspring than do younger mothers. Ciocco (1938) reported the frequency of σ^7 among the first born was .5153; among the fifth or higher was .5124. Novitski (1953) ran a multiple linear regression analysis of the data on 9, 297, 697 white births, and found a highly significant effect of the age of the father but not of the mother. By the path coefficient method Dempster (in the same paper) found that about 23% of the variance in sex ratio was due to direct effect of the age of the father, 1.6% to the age of the mother, and 9% to a joint effect.

There is evidence of a higher proportion of σ among first births born within 18 months after marriage than among first births born later; that is, the proportion of σ children is higher for the more quickly fertile parents. This has been proposed as the explanation for the observation that more males are born in countries during the time they are at war than at other times (Bernstein, 1958).

Cole and Davis (1914) demonstrated that there is competition among the sperm. A rabbit served by two different bucks produced litters in which a majority came from one of the bucks.

Centrifuging was used with rabbits (Lush, 1925) in an attempt to separate the sperms into two size groups but the sex ratios obtained were unaffected. Koltzoff and Schröder (1933) reported that when a current was passed through a physiological solution containing rabbit sperm, part went to one pole and part to the other. From the anode lot, there were 6♀, from the cathode 4♂ 1♀, and from the middle 2♂ 2♀. The writer is aware of no later reports. Attempts to control sex by alkaline or acid douches seem to be negative according to extensive experiments in rabbits and pigs, (McPhee and Eaton, 1943, Cole et al. 1940).

In *Drosophila affinis*, a low-male sex-ratio gene was found by Sturtevant (Morgan, Bridges and Sturtevant, 1925 p. 201) and also by Gershenson (1928) in *D. obscura*. Certain males carried this gene in their X and in all matings with ♀ produced only a few σ or none. Half their grandsons behaved similarly. It is not caused by differential mortality, but must be through some effect on the Y chromosome or on the Y-bearing sperm. A similar gene has been reported in other *Drosophila* species by Sturtevant and Dobzhansky (1936a) and Wallace (1948). Dobzhansky and Sturtevant found that in most meioses an X chromosome divided at both divisions, thus furnishing an X for each of the four resulting cells. The Y passed to half the cells but subsequently usually disintegrated. Occasional sperms received both an X and Y. Rarely a sperm had no sex chromosome or only a Y, these giving the occasional males. For further discussion see White, p. 226-230, 1954. Later studies (Poulson, 1961) have shown the presence of spirochaetes, and that the male-producing larvae are more sensitive and usually do not survive.

Lethals and semi-lethals which are sex-linked affect the sex-ratios. Certain ones kill only homozygous females, e.g. bobbed-lethal, which in the σ is covered by a normal allele in the Y. Redfield (1926) reported a second-chromosome gene which so changes the eggs that viable zygotes come mainly from fertilization by Y-sperm, the offspring being mostly σ .

In interspecific crosses in insects, birds, and in mammals one sex may be absent, rare or sterile. In these cases, it is usually the heterogametic sex which is missing or rare, i.e. in *Drosophila* the male; in Lepidoptera and birds the female. A summary of the sex ratios in interspecific hybrids in mammals has been published by Craft (1938). There is a later publication on mammalian hybrids (Gray, 1958) and one on bird hybrids (Gray, 1958a).

One wide cross in birds, a cross between the ring-dove and the pigeon has been studied by Cole. Painter, through cytological studies of fertilized and developing eggs from this cross, found that the two sexes were formed in equal numbers, but the females failed to survive and the surviving males were actually XX as expected (Painter and Cole, 1943). Riddle (1924) had supposed that those with female chromosomal constitutions had become males by a physiological process of sex-reversal.

In crosses of *D. melanogaster* ♀ with *D. simulans* ♂, the offspring are all ♀; while the reciprocal cross gives all ♂ (Sturtevant, 1921a).

Physiological aspects of sex-expression

Angiosperms

Chemical tests show in general larger quantities of reducing constituents in pistillate sap than in staminate sap. The Manoilov technique is one method used to measure the difference (Loehwing 1938 p. 584). Others have made modifications in the test and also used other tests. These may be of diagnostic value, but conflicting results often occur. Walther and Lilienstern (1934) found differences also in the apical meristems before flowering. Camp (1929, 1932) found that staminate tissues were higher in catalase activity by 50% to 70% in dioecious Morus (mulberry), Papaya, and Arisaema (Jack-in-the-pulpit). Others reported higher temperatures in staminate plants. In Arum the respiratory rate of σ flowers was 10 or more times that of pistillate flowers. There was no difference in pH in certain studies. Hagiwara and Kusamitu (1940) reported the use of potassium chlorate to distinguish the sexes in asparagus. There seem to be highly localized differences in chemical composition in the floral zones.

The following is based on a discussion of the physiology of reproduction in plants by Bonner and Galston (chapt. 17). Usually a certain stage of development must be reached before a plant is capable of flowering. The external factors, length of day, and temperature are important in the control of flowering. Photoperiodism is the response of plants to relative length of day and night. Long-day plants flower at day lengths longer than the critical day length; short-day plants flower at day lengths shorter than the critical day length. Others are day neutral. The number of cycles of exposure to day lengths favorable to flowering which are needed to bring about flowering varies with different species. Bonner and Galston also discuss vernalization, pollination and fruit development.

Schaffner (1927, 1935) reported many experiments in which sex expression was modified by environment, and concluded that sex determination does not occur at the time of chromosome reduction. He listed 12 successive points in the life cycle where sex determination might occur, only two coinciding with chromosome shift. He also stated that organisms with allosomes showed sex reversal as readily as those without. For other references to Schaffner's studies, consult Loehwing's bibliography. It is now recognized that the expression of sex, as is that of many other characters may be modified by environment. This does not change the fundamental concept that the genotype is determined by the kinds of gametes which are united at fertilization.

Animals

Although genes and the chromosome mechanism play a major role in higher species, sex-reversal occurs occasionally. A case of complete sex-reversal was reported by Crew (1923) in which a Buff Orpington ♀ fowl which had produced offspring, changed over to a phenotypic σ which functioned as a σ in production of two offspring.

THE FREE MARTIN. In cattle when twins are born, one of which is male and the other "female", the "female" is frequently sterile, the male usually normal. They arise from the fertilization of two eggs, but the free martin occurs only when there has been a vascular connection between the two embryos. The male hormones apparently transform the ovaries into testis-like structures (cf. Morgan 1926).

In certain lower bisexual species, the chemical environment may determine which sex is to develop or may influence the kind of sex-differentiation (physiology). For example in Bonellia viridis, a marine worm, if a larva settles near the proboscis of an adult female it becomes a σ , if not near a ♀ it develops into a ♀ (Baltzer 1937, cited by Crew 1954).

Biochemical differences

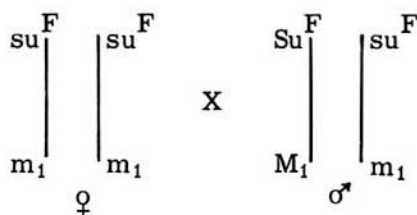
When *Chlamydomonas* is grown on culture media, substances accumulate in the media which affect various stages of sex determination (Kuhn et al. 1944). One of these substances, isorhamnetin (quercetin-3' methylether) has a very specific female sex-determining action on hermaphroditic plants.

The evolution of dioecism

As discussed by Lewis (1942), Allen (1943), Darlington (1956), and Eames (1961) the dioecious breeding system probably has originated from one in which the plants were bisexual.

A system in which the major factors determining sex expression represent alleles at a single locus as in spinach could arise from the monoecious X^mX^m genotype discussed by Janick and Stevenson (1955). Since the dioecious condition assures cross pollination, it might become established if crossing brought about increased vigor. This may help explain the lack of heteromorphic sex chromosomes. If sex were determined by a linked complex of genes, a crossover suppressor system might have a selective advantage. One of these is the heteromorphic XY mechanism such as that in *Drosophila*.

A more elaborate scheme has been proposed by Westergaard (1958) which includes a combination of recessive genes and dominant suppressor genes that may block normal development of staminate or pistillate flowers at a particular step. For example, m_1m_1 or $Su^M Su^M$ would be pistillate and $f_1 f_1$ or $Su^F Su^F$ would be staminate where M and F represent the normal steps in development of male and female organs respectively. If the two loci were completely linked, the 1:1 breeding behavior with the male heterogametic might be represented as follows:



The system requires nearly complete linkage. A non-homologous segment accomplishes this, as in *Melandrium* (Fig. 57).

The genetic schemes by which dioecious breeding behavior was established in maize (see p. 287) depend on the special behavior of certain mutants. The tassel seed mutants suppress anther development and stimulate the development of functional silks in the tassel. The recessive silkless and the barren stalk genes suppress silk and ear development respectively, but have no effect on the tassel.

Concluding statements

As we have seen, the experimental study of sex determination falls into three categories:

1. To determine which sex is heterogametic and to study the breeding behavior.

2. To localize the sex-determining complexes, and to determine the roles of X, Y and autosomes.
3. To explain the end result in terms of gene action.

Some progress has been made in the study of biochemical differences. The isolation of isorhamnetin and its female sex determining action on hermaphroditic plants may have some application to crossing techniques and to plant breeding (Kuhn et al. 1944).

A much better understanding of the problem will be gained if sex expression is regarded as a complex character.