CHAPTER 13

SEX DETERMINATION

Theories of the determination of sex were already numerous in Aristotle's time, and he discussed many of them. His own view was that there is, in each embryo, a sort of contest between the male and female potentialities, and the question of which prevails, that is, the frequencies of the two sexes, may be influenced by many factors, such as the age of the parents, the direction of the wind, and so forth. This idea of a competition between opposing influences has been involved in most theories, down to the present; the current form is described by the term *genic balance.*

The existence of males and females, in approximately equal numbers, continued to intrigue both philosophers and biologists. Thomson in 1908 wrote: "The number of speculations as to the nature of sex has been well-nigh doubled since Drelincourt, in the eighteenth century, brought together 262 'groundless hypotheses,' and since Blumenbach caustically remarked that nothing was more certain than that Drelincourt's own theory formed the 263rd. Subsequent investigators have long ago added Blumenbach's theory to the list."

The discovery of the sex chromosomes and the demonstration of their relation to sex determination, have been described in Chapters 6 and 7. One result of the nondisjunction studies was not pointed out: the X-bearing sperm is not in itself female determining, since it may produce a male if the egg carries no X, that is, sex is determined by the composition of the zygote. This conclusion was confirmed and extended by studies of gynandromorphs. Morgan and Bridges (1919) showed, by a detailed study of a large series of these, that the separate parts of the body of Drosophila are largely independent in their determination, and that the sex of each part is due to its chromosome composition.

Another relation established by Bridges in his nondisjunction work is that diploid individuals of Drosophila with the composition XXY are normal and fertile females, and that those with a single X and no Y (XO) are normal males in appearance—though they are sterile. That is to say, the Y is not the primary sex-determining agent.

A much fuller analysis resulted from the study of triploids by Bridges (1921). These results were based on a remarkable series of parallel genetical and cytological studies, and furnished convincing proof that sex in Drosophila is due to a balance between the number of X's (which have a net female-producing effect) and the number of sets of autosomes (which have a net male-producing effect). Thus, addition of an X to the normal male composition produces a female, while addition of a set of autosomes to the female composition produces an intersex. This conclusion has been fully confirmed by the later finding of a few additional types from the tetraploid females.

Bridges interpreted these results on the basis of the "genic balance" idea that he had deduced early in 1921 from his studies on haplo-IV individuals, which have a recognizable phenotype that differs from the wild type in a number of respects. Bridges pointed out that there is evidence for the existence of numerous genes affecting a given character—some in one way, some in another. Each individual represents the resultant of a particular balance between these variously acting genes. It is unlikely that any given chromosome, or section of a chromosome, will have a set of genes with the same net effect as the whole complement; therefore it is to be expected that duplications or deficiencies will cause changes in this balance and will alter the phenotype—usually to the detriment of the individual, and often with even a lethal effect.

In the case of sex, this interpretation means that there are genes with male-producing effects, and others with female-producing ones; and that the former predominate in the autosomes, the latter in the X. This is, evidently, a form of the competition idea of Aristotle, which had been previously elaborated by Weismann, and by Goldschmidt especially for sex determination. But Bridges based it on more direct experimental evidence and proceeded to use it as a working hypothesis to suggest further experimental approaches. His studies of the effects of different dosages of the small fourth chromosome on the phenotype of intersexes were designed to test the net effect of the genes in this chromosome on the sex of the individual. The results were inconclusive, but the method was used successfully by Dobzhansky and Schultz (1931, 1934) in studying the effects of various fragments of the X. When these fragments were added to intersexes (that is, 2X + 3A + fragment of X), they found that the "inert" (that is, heterochromatic) region was without effect, but that each of the very diverse euchromatic duplications shifted the degree of intersexuality toward femaleness. That is to say, not only does the X as a whole have a net female effect, but the numerous tested portions of it also have such an effect.

Similar tests with duplications for various autosomal segments have given no such clear-cut result, but evidence of another kind indicates that there are several autosomal genes that affect the sex of the individual. Several autosomal mutant genes change diploid females into male-like intersexes (Sturtevant, 1920, for *Drosophila simulans;* Lebedeff, 1934, and Newby, 1942, for *D. virilis;* L. V. Morgan, 1943; Sturtevant, 1945; Gowen, 1948, for *D. melanogaster)*. Four of these genes are recessive, so that the supposition is that their wild-type alleles probably influence development in the *female* direction, rather than in the male direction as the whole set of autosomes does. They do, however, serve to indicate that there are several (many?) autosomal loci concerned.

In 1946 I found that intersexes are produced in hybrids between *Drosophila repleta* and *D. neorepleta,* The analysis indicated that neorepleta carries a dominant autosomal gene which so conditions the eggs before meiosis that two repleta X's are insufficient to produce the full female phenotype and intersexes result—though two neorepleta X's, or one X from each species, produce normal fertile females. Here is evidence for an autosomal gene with the expected male effects; whether or not the unusual maternal effect is generally present in other Drosophila species remains to be determined. It may be noted that this particular gene would not be detected by the usual technique for testing the effects of duplications or deficiencies on triploid intersexes. This gene, since it operates before meiosis and fertilization, cannot be responsible for all the autosomal effects observed in the triploid experiments.

The earlier interpretation of the sex chromosomes of Drosophila, giving the male the formula "XO," meant that the male was haploid for this chromosome, and this was consistent with the facts of sex-linkage. When the work of Bridges and of Metz established that the normal male is XY, it became necessary to suppose that the Y lacked dominant alleles of the sex-linked genes. The study of the XO (exceptional) males by Bridges (1916) showed that, though phenotypically normal, they were sterile. It was shown by Stern (1929) that fertility is dependent on the presence of both arms of the Y, and also (Stern, 1926) that the Y normally carries the wild-type allele of the sex-linked mutant bobbed, so it is not quite inactive genetically. Finally the cytological studies of Heitz and of Painter in 1933 showed that the Y is heterochromatic, like the “inert” right-hand portion of the X.

The work described up to this point has given a reasonably complete and consistent picture of the system of sex determination in Drosophila. It has, however, gradually become evident that this scheme cannot hold in detail for all other forms, even among those in which the normal system can be described as XX, XY.

It was long clear that the Y is not always necessary for fertility of the male, since it is missing in many groups of animals (including Pyrrhocoris, in which the X was first described, and some Diptera—in the same order as Drosophila). The first clearly inconsistent result came from the independent work of Westergaard and of Warmke and Blakeslee in 1939 on Melandrium. This plant is dioecious (has separate male and female individuals) and has clearly distinct X and Y chromosomes, with the female XX and the male XY. The study of induced polyploids and their offspring shows that Y is by far the most important element in the situation: individuals with a Y are male, those without a Y are female. The difference from the Drosophila system was unexpected, but most of us were inclined to minimize it as probably representing a special case. After all, most of the relatives of Melandrium are hermaphrodites, and in the genus itself each sex has clear rudiments of the organs of the other. An earlier indication that the system cannot be widely generalized came from the discoveries by Winge (1922, 1934) in the guppy, Lebistes. Here he showed first that the Y normally carries most of the genes that are responsible for the great variability in the color of the males. He then found that it was possible to produce strains in which the sex-determining mechanism was so modified that the female, rather than the male, was heterozygous for sex. This result was confirmed by Bellamy (1936) for another aquarium fish, Platypoecilus, where two species differ in this way. There is less clear evidence of similar instability of the system in Amphibia, and even in the Diptera there is evidence (Beermann, 1955, on Chironomus; Mainx, 1959, and Tokunaga, 1958, on Megaselia) that different pairs of chromosomes may function as sex-determiners (of the XX, XY type) in different races of the same species.

Finally, it has been found recently that mammals (man, Jacobs and Strong, Ford, *et al.*, 1959; mouse, Welshons and Russell, 1959) resemble Melandrium in that the Y is male determining. Other complications involved are beyond the scope of this book. There is now evidence (Ullerich, 1963) that the Melandrium system applies also to Phormia, one of the blowfly group, where XXY is male. It looks as though Drosophila is a rather exceptional type, even within the order Diptera to which it belongs.

As described in Chapter 6, sex-linkage of the type with the heterozygous female was known in moths and birds before discovery of the type with the heterozygous male, so it was clear from the start of the Drosophila work that there was a type that rested on a different sex-determining mechanism. It was with this type that Goldschmidt made the first attempt at an interpretation in terms of developmental genetics, based on his studies of *Lymantria dispar*, the gypsy moth.

He reported in 1912 on crosses between European and Japanese races of this moth, in which a Japanese female by a European male gave offspring in which both sexes were normal; the reciprocal cross, a European female by a Japanese male, gave normal F_1 males, but the F_1 females were more or less male-like—a condition for which Goldschmidt proposed the term *intersexuality*. After testing these hybrids in various combinations, Goldschmidt concluded that there are two opposing tendencies: F (for femaleness), inherited strictly maternally, and M (for maleness), inherited with the X. One X is insufficient to outweigh F, and FM is a normal XY female; two X's are enough to outweigh F, and FMM is a normal male. In the Japanese race, both F and M are strong; in the European race, both are weak. The intersexes then have a weak F and a single strong M, which is enough to shift the development partially, but not completely, in the male direction.

Goldschmidt's later work with this material was based on crosses with a great many geographical races, especially from Japan, where great diversity in "strengths" of F and M was found. These experiments were summarized in 1933. The original interpretation was confirmed, and greatly expanded and elaborated, in the later papers. It is not easy to evaluate the work. It has certainly been widely cited and acclaimed and has served to focus attention on problems having to do with genes and development. Nevertheless, some of us have serious doubts about it.

One doubt centers on the inheritance of F. This property goes strictly through the female line, without dilution or segregation. At one time Goldschmidt concluded that it was carried in the Y chromosome and exerted its influence on the eggs before meiosis, so that each egg was female in potentiality even if it lost the Y in the polar body. Later experiments seemed to Goldschmidt to disprove this, and he concluded that F is not chromosomal at all, but is cytoplasmically inherited. We are left with no explanation of the earlier experiments that were taken to show that F is in the Y. This contradiction remains unresolved.

The interpretation is in appearance a quantitative one and is often so described, but there are no quantitative data. The numerical values are arbitrarily assigned hypothetical ones: a value of "80" assigned to a single M does not refer to any measured or defined units. The papers contain numerous curves, representing specific hypotheses about the course of development, but these are also arbitrary and not based on any measurements.

The papers contain accounts of a large number of crosses, descriptions, and photographs of many intersexes. One cannot fail to be impressed by the extent of the work—but I confess that I should be more impressed if there had been use of more powerful genetic and cytological techniques, and more attempt to get objective quantitative data.

Another type of sex determination, the understanding of which began to emerge before the discovery of the sex chromosomes or even of chromosomes, occurs in the honeybee and other Hymenoptera and in a few other groups of animals.

Dzierzon, like Mendel, was a Silesian priest. A contemporary of Mendel, he suggested in 1845 that the males of the honeybee arise from unfertilized eggs, the queens and workers from fertilized ones. This view was at first opposed, but came to be generally accepted—especially when modified to state that the females are diploid, the males haploid.

The Hymenoptera are not easy cytological subjects, and the chromosome numbers were long in doubt. In fact, the first clear cytological demonstration of male haploidy and female diploidy was made by Schrader (1920) in the whitefly, Aleurodes (Homoptera). The spermatogenesis of the bee and hornet (Meves, 1904) showed that there is an abortive first meiotic division in which a small enucleate cell is budded off, with the result that each sperm receives an unreduced (haploid) complement of chromosomes.

There were numerous attempts, including one by Schrader and Sturtevant, to bring this system into line with that of Drosophila, but these were abandoned after the work of Whiting and his group on the parasitic Hymenopteran, Habrobracon. In 1925 Whiting and Whiting showed that, if the parents are related, diploid males may be produced in considerable numbers. By 1939 it was shown that there is a single series of multiple alleles (Bostian, 1939; Whiting, 1943)—at least nine members of the series are known—of such a nature that a heterozygote carrying any two different alleles is a female, while any individual with only one (either a haploid or a homozygous diploid) is a male.

This same type of sex determination has been established for the honeybee, but seems not to hold (at least in unmodified form) for some of the other Hymenoptera.

Still another type of sex determination is to be found in the mosses and liverworts, where the haploid generation is sexual. It was shown by the Marchals (1906, 1907) that, in certain mosses, regeneration from the diploid sporophyte (which reproduces by asexual spores) led to the production of sexual gametophytes. These were diploid, and were shown to be hermaphroditic, although the normal haploid gametophytes in these species are either male or female, as are homozygous diploids.

The conclusions suggested were confirmed by the cytological work of Allen (1917) on the liverwort Sphaerocarpos. He showed that the sporophyte has an unequal pair of chromosomes, of which the larger (called X) is present in the (haploid) female gametophytes, while the smaller (Y) is present in the male gametophytes.

Baltzer (1914) has shown that in the marine worm Bonellia sex is determined by the environment, and not by genetic means. This form has perhaps the most extreme sexual dimorphism known, the male being a minute degenerate creature that lives as a parasite in the female. Baltzer has shown that the larvae are not differentiated sexually. Those that settle on the sea bottom develop into females, while those that settle on the proboscis of a female develop into males.

There are many special devices in connection with several of the different types of sex determination, but even a simple catalogue of the more interesting ones would be out of proportion here.

It has sometimes been felt that the determination of sex offered the best opportunity for the study of the manner of action of genes, and the results described here have contributed largely to our understanding in this field; it now appears, however, that it will be more profitable to study simpler situations, and it is to these that attention is now more often directed (Chapter 16).