GENETICS OF SEX DETERMINATION[1]

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Introduction

A complete review of all theories, research, and monographs on the subject of sex determination is a task for a large book. Studies and bibliography on sex determination can be found in the books of Caullery (26), Crew (30, 31) Hartmann (67), Quintanilha (100), Wilson (129), and others. A collection of articles on sex differentiation was edited by Austin (1) and a recent review was produced by Gallien (50). The cytological mechanisms of sex determination have been reviewed recently by Smith (107). Recent books treating the subject with remarkable clarity are Sinnot, Dunn & Dobzhansky (108), Serra (105), and White (124). An excellent review of the genetic balance theory can be found in Goldschmidt's last book (60).

The whole matter was the subject of study even before the genetic or cytologic mechanism of sex determination had been discovered. It is quite amazing to read Weismann's works (120) and see how much he foresaw without sound experimental grounds.

The present article will avoid, where possible, the cytological aspects of sex determination already reviewed by White (124) and Smith (107), and the recent publications on sex differentiation [Austin (1)], and will be limited to the genetic and evolutionary aspects of sex determinations. Some mention of organisms other than insects will be made when necessary for the clarity of the general problem.

Studies on the genetics of sex determination really began with McClung (85, 86) who was the first to discover the meaning of an odd chromosome found in the orthopteran *Conocephalus (Xiphidium) fasciatum* (DeGeer) and suggested that the two types of sperms formed would determine males and females in the offspring. Wilson and his collaborators provided the demonstration for McClung's discovery. According to Stern (110), the German, Henking, in 1891, was the first to detect an X chromosome, in a hemipteran (69). Castle (24) impressed by the 1 : 1 sex ratio suggested that sex could be controlled by a genetic system. Correns (29) postulated that sex would be determined through a back-cross mechanism, males being Mf and females ff.

The merit of the concept that considers sex a quantitative variable character owes its very foundation to Goldschmidt (52 to 55, 60), Bridges (15 to 19), and Dobzhansky & Schultz (37).

[1] The survey of the literature pertaining to this review was concluded in October, 1960.

Definitions

If one wants to study sex determination, he must adopt certain definitions from the beginning. In the past, the concept of sex was strongly prejudiced by the peculiar case of the higher organisms. A very satisfactory and broad meaning was attained by Hartmann (67) and by Serra (105). These authors consider a case of reproduction as being sexual whenever fusion of nuclei (cariogamy) and respective reduction division occur, even if in some cases one of these phases is missing. Two cells are able to fuse if they produce the right kind of copulation or fusion substances. In genetics, sex can be defined as being everything concerned with the combination, recombination, and segregation of genes.

Every living organism is a result of its own heredity and environment. Sex determination is not an exception to this rule. In many organisms, however, the genetic mechanism is so strong and so buffered that changes in the external environment play a very small role. In these cases it is called genotypic sex determination. In other cases, many organisms have their sex determination strongly influenced by environment including their body size, age, or even by the place in the body where the germ cells are produced; such type is called phenotypic sex determination. All classes of intergrades occur between a truly genotypic and a truly phenotypic sex determination; however, for descriptive purposes it is useful to maintain this classification. In monoic plants or in hermaphroditic animals only phenotypic sex determination is found. Of course, the genes for sexuality are there, but chemical substances or temperature of the external environment, the internal environment in the place where anthers, ovaries, and testes are located, age, and feeding conditions determine the sex of the gametes. In dioic plants or in gonochoristic animals phenotypic and genotypic types of sex determination are found, including also all intermediate cases. Goldschmidt (60) is strongly opposed to the use of Hartmann's expression "phenotypic sex determination," claiming that practically all known cases of sexuality can be explained by the genic balance theory, therefore every case is genotypic. We believe that such an expression is quite useful and explanatory, especially in cases where environment has the most important role in shifting sex to female or male, as in the worm, *Dinophilus apatris*. In *D. apatris* the females lay eggs of two sizes, large ones developing into females and smaller ones into males. This happens even in nonfertilized eggs, demonstrating that sex is determined independently of sperm penetration at fertilization [Beauchamp (6)].

The importance of environment in inducing sex was demonstrated by Cleveland (28). He demonstrated that ecdysone (metamorphosis hormone, produced by the insect prothoraxic glands) induces sex in the protozoa *Barbulanympha, Saccinobaculus,* and *Oxymonas.*

Genetic Basis for Sex Determination

The real demonstrations that X chromosomes have something to do with sex determination were provided by Bridges (14) with nondisjunction, and

TABLE I

CHROMOSOME CONSTITUTION AND SEX IN *Drosophila melanogaster* (Meigen), *Melandrium album* (Garcke), AND *Bombyx mori* (Linnaeus)
(A = set of autosomes)

[Frost (46); Goldschmidt (60); Kihara (75); Warmke (119); Westergaard (123)]

Drosophila			*Melandrium*			*Bombyx*	
Chromosome Constitution	Sex	X/A Ratio	Chromosome Constitution	Sex	X+A / Y Ratio	Chromosome Constitution	Sex
2 A XXX	metafemale (sterile)	1.50	2 A XYY	male	1.5	2 A X	male
			2 A XY	male	3.0	2 A XX(−L)	male
3 A XXXX	metafemale (fertile)	1.30	4 A XXYY	male	3.0	2 A XX(−M)	male
			4 A XXXYY	male	3.5	2 A XX(−R)	male
			3 A XY	male	4.0	2 A XX	male
2 A XX	female	1.00	2 A XXY	male	4.0		
2 A XXY			4 A XXXXYY	male	4.0	2 A XY	female
3 A XXX						2 A XXRY	female
4 A XXXX			4 A XY		5.0	2 A XX(L)Y	female
			3 A XXY	male,	5.0	2 A XX(R)Y	female
4 A XXX	intersex	0.75	4 A XXY	occasionally	6.0	2 A XXY	female
			3 A XXXY	hermaphrodite.	6.0		
3 A XX	intersex	0.67	4 A XXXY		7.0	3 A XX	male
3 A XXY			4 A XXXXY	subandroecious	8.0	3 A XXX	male
2 A X	male	0.50	3 A XX	female	∞	3 A XXY	female
2 A XY							
2 A XYY			2 A XX	female	∞	3 A XYY	lethal
4 A XX			2 A XXX	female	∞		
			3 A XXX	female	∞	3 A XXYY	female
3 A X	metamale	0.33	4 A XX	female	∞	4 A XXXY	female
			4 A XXX	female	∞	4 A XXYY	female
			4 A XXXXX	female	∞		

by Morgan & Bridges (88) on gynandromorphs, in both cases using X marked with mutant genes. However, only in the short paper by Bridges (15) on triploid intersexes in *Drosophila melanogaster* Meigen does the theory of genic balance (or, as Goldschmidt adequately points out, genic imbalance) begin to develop. Bridges concluded that sex in *D. melanogaster* depends upon a balance between male-tendency genes, located in the autosomes ,and female-tendency genes of the X chromosomes, Y chromosome being free of sex-determining genes. [See Table I; for detailed explanations of the *Drosophila melanogaster* case see Sinnot, Dunn & Dobzhansky (108) or any other good textbook on genetics.] In Table I we used the expressions "metafemale" and "metamale," as suggested by Stern (111) instead of "superfemale" and "supermale." This scheme works for *Drosophila melanogaster;* however, the general principle that sex depends upon a balance between male-tendency functioning genes and female-tendency functioning genes plus the action of the environment, is correct for all known cases of sexual organisms. We added this word "functioning" since the works of Breuer & Pavan (11, 12, 13), Pavan & Breuer (95), and Pavan & Ficq (96) showed beyond all doubt that not all genes

work at the same time. So, it is possible to conceive the idea of an animal that has gene or genes with inhibiting or exciting effects on the female- or male-tendency genes. The same thing is true for the influence of environment; it can be so strong as to reverse the genetic balance. For instance, Gallien (47) injected ten-day-old tadpoles of *Rana temporaria* Linnaeus with 20 μg of testosterone propionate per individual resulting in 100 per cent males; however, when these tadpoles were injected with 80 μg oestradiol benzoate in oil the result was 100 per cent female at metamorphosis [Gallien (48, 49]. In both cases, environment dominated the genetic influence.

It is not necessary that all male-determining genes be scattered in the autosomes and female-determining genes concentrated in the X chromosome

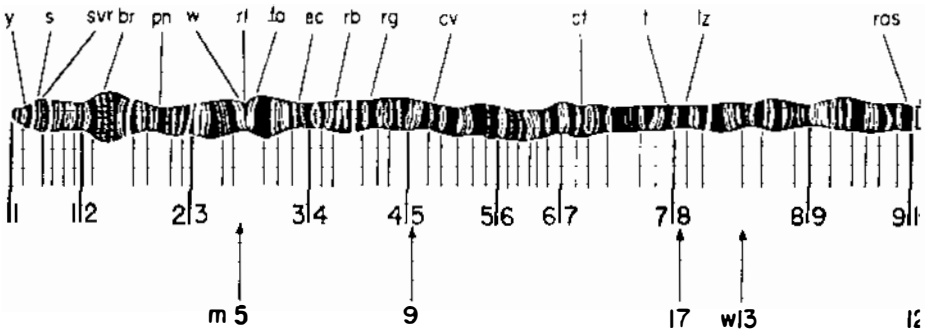


FIG. 1. Cytological map of the X chromosome of *Drosophila melanogaster* showing 10 sections, each one having at least one gene for female tendency (in black) and three sections where no sex genes were found (white). This drawing

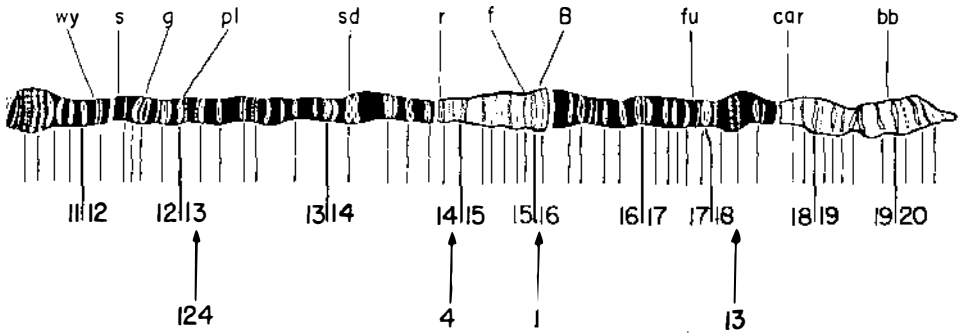
(or vice versa in homogametic male species). In nature it is very common to see general problems like transmission of genic information, accumulation of energy, and irritability with practically the same solution for all organisms, but at the same time the details solved in a multitude of ways. So, the general problem of sex determination has one general solution: the balance among male-tendency genes plus female-tendency genes plus environment. Also, in forms with a low degree of sexuality, sex may be determined by very few genes (as in fishes) or by a great number of them as in *Drosophila, Porthetria,* etc.

Several authors have, even recently, considered the idea that sex is not directly influenced by genes [Swanson (115), p. 466, for instance]. However, year by year, proof is being accumulated that points away from this direction.

Dobzhansky & Schultz (37) demonstrated that there is not just a single

gene for femaleness in the X chromosome of *Drosophila melanogaster* but several of them. Also, they demonstrated that genes for femaleness are not evenly distributed through the whole length of the X chromosome; for instance, there are no such genes close to the kinetochore. The method they used was to study females, males, and intersexes carrying duplications or deficiencies for various sections of the X chromosome. The use of strains producing intersexes for this experiment was indicated because normal sexes are so buffered, their specific balance so distinct, their threshold for change so high, that minor variations would be ineffective. Intersexes are, therefore, good indicators of the presence of sex factors [Dobzhansky (34, 35); Dobzhansky & Schultz (37)].

A tentative map of the X chromosome showing the regions with female-



was based on Bridges (20); Bridges & Brehme (21); especially on Dobzhansky & Schultz (37); Patterson (92); Patterson, Stone & Bedichek (94); and Pipkin (97).

tendency genes was made, in Figure 1, based especially on the work of Dobzhansky & Schultz (37), but also in Pipkin (97), Patterson (93), and Patterson, Stone & Bedichek (94). The zones with female-tendency genes are marked in black and those without female-tendency genes in white; zones are separated by a white bar. To reach this scheme a careful study of Dobzhansky & Schultz's conclusions was made as follows:

Deficiency *y-sc* shifts intersexes toward maleness and duplication (107, 112, 118) (*y-sc-svr*) to femaleness. However, duplication *y-sc-svr* (107) and deficiency *y-sc* when present simultaneously produce a shift toward femaleness, although not as strong as duplication alone. This indicates the presence of gene or genes for femaleness in the segment *sc-svr*. Intersexes with the *y-lz* duplication are more female-like than those with *y-rb* duplication, which indicates the existence of genes for female tendency in the section *rb-lz*. Duplication *y-br* (no. 134) is more female-like than *y-svr*, therefore the segment *svr-br* also carries a female gene. Duplication *y-pn* intensifies

femaleness, indicating female genes in the segment *br-pn*. Duplication for *y-m* interval produces femal-like intersex, just a little more female-like than *y-lz* (some of the intersex with *y-m* duplication layed eggs). All of these conclusions were strengthened by the work of Pipkin (97) which showed female genes in the following genetic combinations: $9L + 2X3A$ (duplicate for segment *y-rb*), $W13L + 17R + 1X3A$ (duplicate for a short segment around *lz*), $m5L + 2X3A$ (duplicate for *y-w* segment), $17L + 9R + 1X3A$ (duplicate for segment a little longer than *w-rb*).

Since no female tendency was found in the interval *v-m* [according to the note added in proof by Dobzhansky & Schultz (37), p. 379], we are led to suppose that the section *lz-v* has genes that somehow improve femaleness. Interval *r-f-B* (dupl. 126) did not show a significative increase of femaleness when duplicated. Lack of female-tendency genes in segment *v-m* was confirmed by Pipkin (97) when translocation *124M* (duplicate for *v-g* interval) did not show an increase in femaleness. Also, $8L + W13R + 1X3A$ (duplicate for short region around *v*) did not differ from control. Furthermore, $8R + 2X3A$ (duplicate from *y* to little over *v*) produces intersex practically identical to *W*13R2X3A (duplicate from *y* to a little before *v*). The lack of female-tendency genes in segment *rfB* was, in part, confirmed by Pipkin (97) when flies $1L + 4R + 1X3A$ (duplicate for a segment a little longer than *rfB*) did not differ from controls.

Intersexes containing either *y-pn* (dupl. 136) or *fu-bb* (dupl. 138) intervals are sterile. However, duplicate 100 (*y-pn, fu-bb*) produces a fertile individual which shows the additive and quantitative character of sex-determination mechanism. Furthermore, if the *fu-bb* segment showed female tendency, the segment *car-bb* did not show it; therefore, the section responsible for femaleness is *fu-car*.

Duplication of the region *s-car* is much more female-like (although sterile) than is the duplication of *r-bb* (dupl. 138), indicating female-tendency genes in segment *s-g-r*. This confirmed Patterson's (92) findings of a strong female-tendency gene (or genes) in the *g-f* region, later on limited to the region *wy-g* (wavy-garnet) by Patterson, Stone & Bedichek (94). Patterson (93) refined this limitation to the small region *g-pl* (garnet-pleated, between sections 13A2 to 13A6). However, Pipkin (97) demonstrated that there is no such thing as a "primary" sex gene in that region.

Therefore, ten regions of the X chromosome, namely, *y-sc, sc-svr, svr-br, br-pn, pn-rb, rb-lz, lz-v, m-s, s-r, fu-car,* have at least one gene for female tendency; three regions, *v-m, r-B,* and *car-bb,* do not have such genes.

Goldschmidt (60) suggests that the female tendency is produced by the intercalary heterochromatin. His statement is based on the fact that the part of X chromosome to the left of section 17 has more feminizing influence than that to the right [Pipkin (97)], and that almost all intercalary heterochromatin is found in that left section. Arguments against such a hypothesis are, however, very strong. Pavan and his collaborators (44, 95, 96) demonstrated that heterochromatic regions of the X chromosome (and of the

autosomes too) in *Rhynchosciara angelae* Nonato & Pavan and *Rhynchosciara milleri* Pavan & Breuer are not in the same place when different tissues are considered; for example, heterochromatin from salivary glands have a quite different disposition from those of the Malpighian tubules. Furthermore, there is intercalary heterochromatin in the intervals where no female-tendency genes were found.

Goldschmidt (60) refuses to consider all mutant loci which can produce a shift in sex as sex determiners, and calls them sex modifiers.
tion only introduces confusion in the balance theory rendering almost impossible an explanation of several cases (bees, for instance). We consider as an indirect demonstration of wild type sex-tendency genes in the autosomes the instance when their mutants confer maleness to females. As Stone (112) points out, neomorphic mutations, that is, those with effects unrelated to their normal allels, are rare. Some of the genes in the autosomes affecting sex are [Gowen & Fung(66)]:

D. simulans Sturtevant: Second chromosome, a recessive gene that shifts normal females to intersexuals [Sturtevant (113)].

D. virilis Sturtevant: Third chromosome, a recessive gene *(ix)* converts females into intersexuals [Lebedeff (78, 79)].
Second chromosome, a dominant gene converts diploid females into intersexuals [Newby (90, 91)].

D. virilis Sturtevant: Third chromosome, a recessive gene (ix) converts females into intersexuals [Gowen & Fung (66)].
Third chromosome, a recessive gene (transformer locus), "*tra*" which, when homozygous, transforms diploid females into sterile males [Sturtevant (114)].

Gowen & Fung (66) carried out an experiment that provides one more strong point against the Goldschmidt hypothesis. They showed that gene *Hr* has a dosage effect in triploids and that both the mutant *Hr,* and its wild type hr^+ counterpart, influenced both the embryological and phenotypical sex. Consequently both *Hr* and hr^+ are correctly regarded as true major sex genes. It is interesting to cite that Pipkin (98, 99) could not demonstrate male regions in chromosome II. Therefore, most of the genes for male tendency in *Drosophila melanogaster* are to be found in the third chromosome (like Hr^+).

Kelstein (72a) suggested that a male sex-determining region exists between 89 A and 94 A on the salivary map of chromosome 3 of *Drosophila melanogaster* [Pipkin (99a)]. However, Pipkin, using aneuploidy of long regions of chromosome 3, found no shift toward intersexuality in hypertriploid females. These two different results may suggest different action of the male genes which may not have an additive action as that attributed to female genes. If this be the case, the conclusions of Kelstein, who used deficiencies, are more reliable than those of Pipkin who used triploids.

Other organisms furnish further evidence for the existence of male tendency genes in autosomes, being responsible for the sex balance. Males

of *Lebistes reticulatus* (Peters) are XY and females XX, male-tendency genes existing in one extremity of the Y chromosome and in some autosomes, female-tendency genes existing in the X chromosomes and perhaps also in autosomes. Winge (130), selecting among XX females, was able to concentrate enough male genes in one autosome to change it into a sexual chromosome. Of course, crossing over could not be prevented between these two new "sex chromosomes," and such gene constellation could only be maintained through severe selection. To consider these male-tendency genes as "intercalary heterochromatin" would mean to confer to heterochromatin the same properties as normal genes. Winge's experiments provided a good explanation for Gordon's (61, 63, 64) findings that natural races of *Xiphophorus maculatus* having in some places heterogametic females (strains from Belize River, British Honduras) and in others homogametic females (four rivers that drain into the Gulf of Mexico, namely, Jamapa, Papaloapan, Coatzacoalcos, and Grijalva).

The case of the moth *Porthetria dispar* (Linnaeus) [= *Lymantria dispar* (Linnaeus)] studied by Goldschmidt since 1911, has been recently reviewed by the same author [Goldschmidt (60)]. This species is geographically divided in many races, inhabiting Europe, northern Africa, and northern, central, and eastern Asia. Sex determination can be explained under the balance theory admitting female-tendency genes in the Y chromosome and male-tendency genes in the X, these genes being of different strength from race to race. Within each race the balance is maintained and produces 1 ♂ and 1 ♀, but by crossing different races this balance is upset and all grades of intersexes appear. Because of Goldschmidt's paper of 1934 and others, his working hypothesis that femaleness is carried in the cytoplasm and maleness in the X chromosome is frequently cited. However, since 1942 (59) and more directly in his work written in 1955, he states his belief as the one presented above; that is, intersexes develop when strong *F*'s and weak *M*'s or vice versa, are put together. In silkworm (*Bombyx mori*) the situation is the same (see Table I) with the difference that no intersexes have been found in any inter-racial crosses [Kihara (75)] nor in any balance between different degrees of ploidy. Goldschmidt (60), based on the findings of Westergaard (122) in *Melandrium,* suggests that autosomes of Lepidoptera may have sex genes. The plant *Melmandrium album* has been extensively studied as far as its sex determination is concerned, with the aid of polyploids and polysomy. Revisions of the subject are found in Warmke (119), and Westergaard (123) (see Table I). X chromosomes possess female-tendency genes and Y chromosomes male-tendency genes. With selection it is possible to increase femaleness, which indicates the presence of some sex genes also in the autosomes [Westergaard (122)].

Parthenogenesis

The case of thelytokous parthenogenesis usually fall directly in the XX-XY case of sex determination. Because of some class of apomixis, the

egg becomes *2n* and, therefore, the egg is XX (or XY[2] in Lepidoptera) and the sex is female. Hundreds of cases are known with such a mechanism [reviewed by Suomalainen (109)]. Some of them appear to have arisen very recently as in *Drosophila mangabeirai* Malogolowkin [Carson, Wheeler & Heed (23); Murdy & Carson (89)] where thelytoky has already suffered such modifications that individuals are characterized by having a fixed genotype, while in *D. parthenogenetica* Stalker eggs, a great many cytological abnormalities are found.

Tucker (118) studied the cytology of thelytoky in honey bees (*Apis mellifera,* Linnaeus). There, thelytoky is very rare. Mackensen (83) estimated that about 1 per cent of non-fertilized eggs develop into females. However, in *A. m. capensis* (Escholtz), workers of queenless, weak hives lay eggs that develop into females. I crossed queens of *A. m. capensis* with Italian drones (*A. m. ligustica* Spinola) using two marking genes to determine whether or not higher percentage of thelytokous females would be obtained among the brood of F_1-laying workers. This has not yet happened even after a backcross to *A. m. capensis,* which shows that the thelytoky complex genes are so stable and so balanced, that even two crosses toward *A. m. capensis* rendered it impossible to obtain stabilized thelytoky.

Arrhenotoky exists in several groups of animals [see Doutt (38); White (124)] and can have a different cytology from group to group. In the majority of Hymenoptera, males originate from unfertilized eggs (haploid) and females from fertilized eggs (diploid). On the other hand, Brown & Bennett (22) discovered in the diaspine scale, *Pseudaulacaspis pentagona* (Targioni-Tozzeui), that mating is required for the production of any offspring either male or female. Using chromosomes broken by x-rays as markers, they demonstrated that the eliminated set is of paternal origin. In bees, males develop from unfertilized eggs so, in the end, males from both Apoidea and Coccoïdea have only one set of chromosomes of maternal origin. Therefore, both males and females apparently have the same genic balance.

Explanation of sex determination by arrhenotoky has been the subject of several speculations. Goldschmidt's old theory (56, 57, 58) was used to explain the case of *Apis mellifera* in which chromosomes would bear genes for femaleness while cytoplasm would carry maleness elements. According to this theory, one set of genes for femaleness would not be enough to outweigh factors for maleness and, therefore, haploids would be males. Two sets of chromosomes, however, would be sufficient to dominate cytoplasmic maleness, so diploids would be females. Somewhat against this theory are the experiments of Schnetter (104) and DuPraw (43) who were able to exclude 21 and 25 per cent of the egg length without any

[2] We prefer the system of writing XY females instead of WZ. In fact, in the fish *Xiphophorus maculatus* Günther, where both hetero- and homogametic females occur, Gordon (62) demonstrated that X and W are homologous; therefore the introduction of W in the nomenclature in unnecessary.

sexual modification of the emerging insect. The ideas of Goldschmidt on sex determination were criticized by Winge (131), who showed that his assumptions were unnecessary, and was able to explain the *Porthetria* (=*Lymantria*) case in the orthodox way.

Whiting (125, 126, 127) developed the multiple alleles theory to explain the case of *Bracon hebetor* Say (= *Habrobracon juglandis* (Ashmead)). He found that females of *Bracon* are heterozygous for one pair of sex alleles as: X_1X_2, X_4X_3, X_7X_4, etc., while haploid males are hemizygous, as: X_1, X_2, X_3, X_4, etc. The combinations X_1X_1, X_2X_2, X_3X_3, result in weak, low-viability, diploid males. Mackensen (84) suggested that the same occurs in *Apis mellifera,* the homozygous for one X-factor male larvae dying within four days after egg hatching. This multiple-alleles theory does not explain all cases among the Hymenoptera, as, e.g., *Melittobia* where endogamy is the rule [Whiting (128)], and *Telenomus fariai* Costa Lima [Dreyfus & Breuer (39)]. I found also that it does not explain the case of the meliponid bee, *Melipona scutellaris* Latreille, that was brother-mated for six generations without any sign of 50 per cent inviability. However, Rothenbuhler (101, 102) found sound evidence to confirm Mackensen's (84) hypothesis for *Apis mellifera.* The number of these sex alleles was estimated to average 13 by Laidlaw, Gomes & Kerr (77) in a panmitic population.

Cunha & Kerr (33) proposed a new working hypothesis to explain arrhenotokous parthenogenesis. They said that it could be explained assuming a series of male-tendency genes *m* and a series of females tendency genes *f,* scattered through several chromosomes. The effects of *m* would be the same in hemizygotes *m* as in diploids *mm,* and the effect of all *m*'s may be represented as *M* in both kinds of individuals. The effect of *f,* would be cumulative, and, therefore, would be *F* in the haploid set of *f*'s and *2 F* in the *ff* sets of diploids. So, the sex would be determined by the equations $2F > M$ = female, $M > F$ = male. Mutations for cumulative effect when occurring in *m* genes would produce *2M* effects. Such mutations would have deleterious effects because they would reduce the normal number of females and therefore would act as semilethals. According to Cunha & Kerr, mutations for noncumulative effects could be viable in the *f* genes since the different alleles would continue to show *2M* effect in heterozygotes. The case of *Bracon* can be explained along these lines. The *x*-genes could be a pair of *f* genes which have lost their cumulative effect for femaleness in the homozygous condition but still have it as a heterotic effect. It is interesting to notice, along these lines that triploid Bracon $X_1X_1X_2$ are females [Inaba (70); Torvik-Greb (117)] which is in accordance with this last explanation and is not in accordance with an inhibitory-effect hypothesis or a maleness-effect hypothesis of X_1X_1.

It is also interesting to see that x-ray treatment of females, *Pseudaulacaspis pentagona,* with 1285 r, 3000 r, and of males with 5000 r diminished

the normal proportion of females (about 2:1) to, respectively, about 1:6, 1:4, 1:3 [Brown & Bennett (*22*)]. This is exactly what is expected according to the genic balance hypothesis just mentioned.

Gans (51), Kerr & Kerr (74), and Cunha (32) demonstrated the existance, in two species of *Drosophila,* of genes with sex-limited effect in the X chromosome (the X chromosome is haploid in males and diploid in females). Hymenoptera with all chromosomes in the haplo-diploid system should have many more of these genes and some of them with a role in sex determination. Kerr (73) demonstrated several such genes in populations of stingless bees, but more refined demonstrations are still to be made.

Sex Chromatin

Barr & Bertram (2) discovered, in nerve cells of the hypoglossal nucleus of the cat, a Feulgen-positive chromosomic structure usually adhering to the nucleus membrane which they named sex chromatin. They found it present in female cats and absent in males, so they assumed sex chromatin to be the same as the hetero chromatin of the XX chromosome pair. Barr (3) found that this sexual chromatin is larger in females than in males, and is found generally in almost all mammals.

Castro & Sasso (25) observed that in cells from brain, liver, and pancreas the sexual chromatin (called chromolema by these authors) is present in twice the quantity in females than in males. However, in other cells (skin, testicles, uterus, blood) this sexual chromatin of females is several times bigger than in males, in whom it is hardly noticed, so they concluded that this is attributable to the function of the female genes in the X chromosome. This conclusion is based on the works of Breuer and Pavan which demonstrated the enormous growth in volume in small sections of chromosomes of *Rhynchosciara angelae* and *R. milleri* attributable to the genic action. Russell, Russell & Gower (103) and Welshons & Russell (121) demonstrated that the 2A/X*O* mice are female and not male. Jacobs & Strong (71), Ford and collaborators (45) demonstrated that XXY is intersexual in men. This is unexpected if one considers the genes in balance to be only those of the X chromosome and those of the autosomes.

Based on Castro & Sasso (25), and on the above authors, we can advance a hypothesis to explain sex determination in rat and in man, and possibly some other mammals, as follows: (*a*) The Y chromosome has genes that produce an inhibitory effect on the female-tendency genes of the X chromosome. In the absence of Y, female genes of one X chromosome appear to be enough to outweigh the autosomal male genes; (*b*) As Castro & Sasso (25) postulated, the size of the sexual chromatin may be due to the function of the ♀ genes in the X chromosomes. Cells of the human liver and pancreas that are not strongly sexually differentiated, have sexual chromatin in the proportion of two in females to one in males. However, cells of sexually differentiated tissues, like testicles, uterus, skin, and blood, have

proportional size more than 10:1 (female to male), giving, in that way, a demonstration of the gene function in sex differentiation.

Smith (106) found sex chromatin in somatic cells of larval silk glands of females of the spruce budworm, *Choristoneura fumiferana* (Clemens), where females are XY and males XX. Males do not present such structure. Kosin & Ishizaki (76) found that female *Gallus gallus domesticus* had sexual chromatin but that males do not. The cells that presented more clearly the sexual chromatin were those of the feather epidermis which showed sex chromatin in 50 per cent of the cells while males showed it in only 5 per cent of the cells. For many, these two facts constituted a puzzle, because they expect to find sexual chromatin in the XX males, and not in the XY or X*O* females. However, this supports Castro's and Sasso's gene-function idea. If femaleness genes act in the two X chromosomes of an XX female mammal, they should also act in the autosomes of an X*O* bird or in the Y of an XY Lepidoptera. This seems to be the case.

EVOLUTIONARY FORCES AFFECTING GAMETOGENESIS

Many cytologists have been amazed by the constancy of the meiotic mechanism in the tremendously variable world of diploid organisms. The reason is that there is only one major problem to solve: reduction of chromosome number. However, after meiosis different methods are followed for the maturation of the haploid cell in becoming a pollen, a sperm, an oösphere, or an ovule. In the case of sperm, for instance, they must be prepared to live only a few hours (Ephemerida), a few days (*Drosophila*), several months (*Polistes, Bombus*), or many years (*Atta, Apis, Melipona,* etc.). Some must be adapted to an aqueous environment, others to a non-aqueous one, still others to acid, to neutral, and so on. Therefore, natural selection provides sperms adapted to each circumstance so that, spermiogenesis is variable from organism to organism. However, some effect of this selection goes back to meiosis. In certain *Diptera* and in *Ascaris,* the somatic cells have smaller amounts of chromosomic material than germline cells. Either entire chromosomes as in *Sciara coprophila* Lintner [Du Bois (40)], or the extremities of a chromosome as in *Ascaris megalocephala* Cloquet (= *Parascaris equorum* (Goezc) [Boveri (10)] make the difference. Usually the differentiation takes place in early embryonic division, where there is elimination of the chromosomic material. In other organisms there is abortion of half of the meiotic nuclei, so that the cytoplasm can be thoroughly used by the remaining cell. This is the case of more than 20 species of bees we have examined, and also in all known *Sciara* [White (124)]. Boveri (9) suggests a correlation between the existance of extra germ-like chromatin and the structure of eggs and sperms. Scholl found that a parthenogenetic strain of *Pseudosmittia arenaria* Strenzke with yellow chorion has supernumerary chromosomes, and a strain with brown chorion has none [see Beermann (7)].

It might be that the abortion of a nuclear spermatid bud in the second division of bees could be attributable to selective advantage conferred upon sperms containing more cytoplasm, more reserve, more ATP, for instance. Sperms with more reserve substances might live longer in the female spermatheca. Such a trend of selection probably reached a maximum in *Apis mellifera*. Jaycox (72) kept undiluted semen containing many actively motile bee spermatozoa at 35°C in flame-sealed capillary tubes for 22 days, and a few motile spermatozoa for 34 days. Taber & Blum (116) obtained fertile eggs from queen honey bees inseminated with undiluted sperm stored at room temperature for 68 days in sealed glass tubes 2 mm in diameter.

Torvik-Greb (117) provided good evidence that elimination of a small cytoplasmic bud in the first
haploid number but of a genetic constitution, because diploid males of *Bracon* also eliminate that small bud [White (124)].

Several examples of genes affecting gametogenesis are known, as, for example: the "sex ratio" genes found in the X chromosome of several *Drosophila* species [see bibliography in Dobzhansky (36)], a gene that prevents crossing over and promotes non-disjunction in *D. melanogaster* [Gowen (65)] and many others. However, the most striking gene affecting some part of spermiogenesis, was discovered by Dunn in the mice *Mus musculus* Linnaeus [Dunn (41, 42)] in the locus T. Animals $+/t$ have a selective advantage on $+/+$, but t/t is lethal. However, males $+/t$, when mated to $+/+$ females, produce a proportion of 96 per cent $+/t$ individuals against 4 per cent $+/+$. This indicates that sperms t have an enormous selective advantage over $+$ sperms. Based on this case, Dunn described a new evolutionary force able to change gene frequency, and called it "male segregation ratio." It is very likely that genes of this kind in several species of insects are much more frequent than is usually thought.

All characteristics of an organism's life are important in fitting
a certain environment; the same is true of the sex ratio. Many genes have been discovered that control sex ratio. Therefore, in many insects sex ratio is altered by diverse mechanisms, but primarily by genic influence. In the coffeeborer [*Hypothenemus hampei* (Ferr), Coleoptera, Ipidae], sex ratio varies between 1 ♂ :5.7 ♀ to 1 ♂ :40 ♀. Bergamin & Kerr (8) found the elimination of a sex chromosome the cause of such biological fact.

Evolution of Sex Determination

Sexuality in insects is so complex and so old, that it cannot be used to explain the origin of sexuality. For understanding such origin, we must study the microorganisms. Normally, sexual microorganisms have a long haploid life and a very short diploid life, immediately followed by reduction division. Before sexuality arose, all variations, all conquests of new ecological niches had to be accomplished through mutations. The first mu-

tations that allowed fusion of cells and consequent combination and recombination of mutants may have been of the type still seen in some bacteria and viruses. Until 1946, bacteria were believed to be completely asexual organisms. However, Lederberg & Tatum (80) showed that in *Escherichia coli* (Magula) Castellani and Chambers, there are strains that are F^+ and strains that are F^-. The F^+ strains when mixed with F^- strains produce recombinations in the frequency of only 10^{-5} to 10^{-6} that of the parental population. Two mutant strains of F^+ were isolated (Hfr) in which the frequency of recombinants was 20,000 times higher than in the equivalent $F^+ \times F^-$ cross [Cavalli (27); Hayes (68)]. A study of the conjugation, genetic recombination, and subsequent division of *E. coli* was done by Wollman, Jacob & Hayes (132); among other details, they found that the whole sexual process in that bacteria takes about 140 minutes and that it is an energy-requiring process. The advantage of sexual reproduction is the enormous variety of genotypes that an organism puts at the disposal of natural selection in its fight to survive, to compete with other species, and to conquer new ecological niches. In others words, major advances of evolution became possible only after sexual reproduction had evolved because a greater proportion of gene combinations could be explored by life, and consequently numerous, well-adapted organisms would arise [Dobzhansky (36)]. In spite of all evolutionary advantages of sexual reproduction, Lederberg *et al.* (81) found, among 650 independently isolated strains of *E. coli,* that only 4 per cent of them would mate. A probable explanation for this might be disclosed by the findings of Miyake & Demerec (87) and Baron, Carey & Spilman (4, 5) that demonstrated that strains Hfr of *E. coli* also mate with strains of *Salmonella* and *Shigella* (both belonging to the Enterobacteriaceae). Apparently, this bacteria has not yet developed a good isolating mechanism and this may explain the low frequency of mating strains. These facts indicate that the first step toward sexual individuals is a mutation for conjugation. The next step, after rudimentary sexuality has been established, is to develop a way to protect the well-adapted organisms from disintegration, that is, to develop an isolating mechanism. In higher organisms, more genes may have mutated and become involved in sex determination. Usually the genes for one sex are gradually grouped in one chromosome [for evolution of sex-determination chromosomes, see chapters XV and XVI of White (124)] which, through selection, slowly stops recombination with its homologous if it contains genes for the determination of the other sex. Progress of evolutionary forces can also cause a strong differentiation in the Y chromosome. Lima-de-Faria (82) found that sex chromosomes of male *Melanoplus differentialis* (Thomas) synthesize DNA later than do the autosomes of the same cell.

For the protozoa, the arising of new individuals is not related to the death of the previously existing. The growth is done by division, and so the two new beings are identical, no one being older or younger. Therefore,

the thousands and thousands of microorganisms are as old as the species itself. Natural death is, therefore, a property shared only by multicellular beings [Weismann (120)] and also for some unicellular ones which have already evolved sex, like *Paramecium*. The metazoa lost this ability of being eternal because of its development of cell diversity. Grossly, cells can be classed as somatic or germinal. Only this last group retains such property as the immortality of the unicellular beings. Life span is an adaptation of the species to the environment and its unlimited continuation would mean for evolution an unnecessary luxury. Death is opportunistic, because it eliminates from within a species the old, incapable, defective individuals that are occupying ecological niches which otherwise would be occupied by better fit, younger specimens [Weismann (120)]. In more genetic words, senile individuals diminish the general adaptive value of a species. Therefore, evolution of sex after a certain level is parallel to the evolution of death, both working together to improve the fitness of metazoa. The way found in nature to preserve the eternity of life and fitness of species was that of separating germ cells from somatic cells. This solution was developed in such an array of forms and methods that it is keeping thousands of biologists busy trying to understand all its intricacies.

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