PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Genetic Basis for the Determination of Sex [and Discussion]

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Genetic basis for the determination of sex

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Contents

An introductory review is given of some general aspects of the genetic basis for the determination of sex, with particular reference to vertebrates. The Weismann–Fisher view is that sex arose because it was of selective advantage. The bisexuality of vertebrates is not necessarily 'best' for the group in terms of selective advantage; however, the very simplicity of the bisexual system may have been advantageous. The common near-equality in the numbers of the sexes is explained by the Darwin–Fisher 'automatic 1:1 tendency', and Fisher's concept of parental expenditure can explain deviations from equality and also changes in the ratio during embryogenesis. A direct selective advantage can be argued for the 1:1 ratio—or, more generally, for that ratio which maximizes the chance of a successful encounter of the sexes. Possible modes of gene action affecting sex ratio are discussed. A new assessment is made of the identity of the heterogametic and homogametic sexes in vertebrates, the conclusions for Amphibia deviating from those in some of the literature. It is maintained for vertebrates in general that the 'odd' sex chromosomes (Y or W) of a species determine strongly in the direction of one gonadal sex. Responsibility for determining the opposite sex must lie between the autosomes and the 'even' chromosomes (X or Z), but there is little evidence that the 'even' chromosomes are specifically implicated. Sex differentiation may not be controlled by a limited number of sex-determining genes, and the ultimate units may be aggregates of the genetic material larger than genes. The possibility of controlling sex ratio at conception by altering the proportion in which X- and Y-bearing spermatozoa fertilize eggs is discussed in relation to the more general question of whether the phenotype of a developing or mature spermatozoon can be affected by its own haploid genetic content.

I have felt that this first lecture of the Discussion should be introductory, even at the risk of detailing facts known to almost everyone, that I should try to bring out general points that are sometimes lost sight of in specific fields of research, and that I should avoid anticipating what later speakers have to say. The choice of topics is somewhat personal; they concern on the whole, animals rather than plants, vertebrates rather than invertebrates, and mammals rather than non-mammals. Unashamedly, I am going to use teleological statements, attributing to Nature purpose, striving, and so on. Teleological statement is often much easier to understand, and a 'true' teleological statement can always be translated into the more formal scientific categories of material and efficient causes. I have justified this approach elsewhere (Beatty 1965). The topics are arranged as a sequence of seven questions.

Why does sex exist at all?

The genetical answer, in brief, is that sex is thought to offer selective advantages in the struggle for existence, and that tendencies towards sexuality were seized upon by natural selection and perpetuated and elaborated thereafter throughout evolution. This was visualized by August Weismann (1887). In his own words: 'We must attempt to explain the reason why Nature has insisted upon the rise and progress of sexual propagation.' And later: '. . . we are led to the conviction that sexual propagation must confer immense benefits upon organic life. I believe that such beneficial results will be found in the fact that sexual propagation may be regarded as a source of individual variability, furnishing material for the operation of natural selection. I believe that sexual propagation has become prevalent among the higher organisms for the purpose of conserving and multiplying that individual variability which owes its first origin to the Protozoon condition of such higher organisms.'

These ideas were brought into the context of modern genetics by Fisher (1930): 'A consequence of sexual reproduction which seems to be of fundamental importance to evolutionary theory is that advantageous changes in different structural elements of the germ plasm can be taken advantage of independently; whereas with asexual organisms either the genetic uniformity of the whole group must be such that evolutionary progress is greatly retarded, or if there is considerable genetic diversity, many beneficial changes will be lost through occurring in individuals destined to leave no ultimate descendants in the species. In consequence an organism sexually reproduced can respond so much more rapidly to whatever selection is in action, that if placed in competition on equal terms with an asexual organism similar in all other respects, the latter would certainly be replaced by the former.'

This is, therefore, a powerful genetic argument saying that sex exists because it has been of selective advantage during evolution. From the cytological point of view, the essential job of sex is to allow for new arrangements of genes for natural selection to operate upon. This happens at two levels. The random assortation of maternal and paternal chromosomes means that whole blocks of genes can be shuffled, as it were, into the hybrid. And the mechanics of meiosis allows for shuffling within the chromosomes by means of crossing-over.

Why are there two sexes in vertebrates?

I do not know of any detailed body of thought on this question. I remember a recent lecture by Professor J. H. Burnett in which he estimated that the global population of the basidiomycete fungus *Schizophyllum commune* contains potentially about 400 000 different sexes, in the sense of that number of different mating types, and that from his point of view the bisexual system of mammals and other animals was a simplified specialization of comparatively minor interest. But the vertebrates *have* adopted a bisexual system. Does this mean that bisexuality in this group has some selective advantage? Here I will digress for a moment to illustrate the dangers of retrospective justification of evolution—the dangers of assuming that because the individual characteristics of a species exist, therefore each individual characteristic must *in itself* have had a selective advantage. For example, the isopod crustacean *Niphargus balcanicus* has sometimes a symmetrical black and white external pattern, the beetle genus *Leptodirus* has a golden brown colour, and certain races of the amphipod crustacean *Asellus aquaticus* show an extreme variability in the pattern and depth of colour of different individuals. From a simple

point of view one might have argued that because these colours and patterns exist in the species, therefore they must be of direct selective advantage. But when I now reveal that all these animals are restricted to the total obscurity of the cave environment, it will become obvious that their external colour and pattern *in themselves* can have no immediate selective advantage whatsoever. Another example is the well-known one of sickle-cell anaemia in man, a genetic trait with a high gene frequency in some populations indigenous to malarial districts. The 'character' itself is actually disadvantageous, being a homozygous lethal. Natural selection has struck a balance; the relatively few homozygotes are tolerated because of the compensating and overriding advantage that the relatively more numerous heterozygotes possess a degree of resistance to malarial infection.

Leaving this digression, and returning to the main question; it would be dangerous to assume that bisexuality in itself is 'better' for vertebrates than polysexuality (more than two sexes). One is free to imagine that polysexuality might offer a more pliable, sophisticated set of opportunities to natural selection. On the other hand, one could speculate as follows: if we agree that sexuality itself is of selective advantage, then the simplest way of having more than one sex is to have two, and the chromosomal mechanism can be relatively simple. Therefore, on the grounds of biological economy, natural selection might have chosen the simpler situation of bisexuality. However, it is quite possible that one is asking a non-question. In higher vertebrates, at any rate, it may be that bisexuality has always been so embedded in the biology of the group that a remodelling of the genetic structure as a polysexual system would be very difficult; in this group, natural selection might never have had an opportunity of 'choosing' between bisexuality and polysexuality.

Why are the sexes commonly equal in number?

Here there has been a great deal of thought concerned with establishing facts and with interpreting them (e.g. the review of Edwards 1962).

First, there is what I will call the Darwin–Fisher 'automatic 1:1 tendency'. The argument was stated explicitly by Darwin (1871, vol. 1, p. 316). It was developed by and is sometimes attributed to Fisher (1930) and may be put as follows. Suppose that you are one person in a population which happens to have evolved a sex ratio to the point when there are about 1000 men and about 10 women in each generation. Now suppose that you happen to have an hereditary tendency to produce an even greater proportion of sons. These sons will have a small chance of being able to marry and to pass on your special male-producing genetic trait to future generations. But if you have a genetic tendency to produce a relatively high proportion of daughters, each daughter will have an excellent chance of marrying and of passing on your female-producing trait. So, in short, the succeeding generations will see a relative accumulation of tendencies to produce more daughters—that is, there will be an increase in the relative numbers of the rarer sex. There is an obvious equilibrium when neither sex is rarer than the other; in other words, when the numbers of the sexes are equal. This, then, is a genetic argument showing why the numbers of the sexes should tend to be equal. Now this is really an argument for a 1:1 tertiary sex ratio, the *effective* sex ratio when reproductive ability is acquired, which in man is at much the same age as when independence from the parents is acquired. Built into Fisher's original exposition was a factor of biological economy that he called *parental expenditure*; that is, the biological time and trouble incurred by parents in gestating and rearing offspring to reproductive age. This had two interesting aspects. The first is as follows. Suppose

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

that one sex, say the male sex, has a high mortality towards the end of the rearing period, so that death of an offspring means a complete loss of all the biological expenditure that went into its upbringing. Natural selection would then be expected to make a compromise; it would try to evolve a sex ratio that gave fewer of the biologically more costly sex, but as against this there would still be the automatic tendency towards a 1:1 ratio just described. Some kind of a balance would be expected, and the concept of parental expenditure would therefore allow for tertiary sex ratios that depart from a 1:1 ratio.

The second interesting aspect in considering the factor of parental expenditure is that it offers an explanation of different sex ratios at different ages. Suppose that one sex, say the male sex, has an undue mortality during very early embryogenesis. The loss of biological capital is not very great, and natural selection could make up the numbers of eventually viable male embryos without undue biological cost simply by arranging to have more male embryos. In this way, one could have an initial excess of male embryos compensating for the later loss and giving in the end the automatic 1:1 ratio.

Although this automatic tendency towards a 1:1 sex ratio explains the common near-equality of the sexes as a consequence of a selection process that is of obvious advantage or disadvantage to the individuals whose contribution to future generations is either favoured or else disfavoured, it does *not* say that a 1:1 ratio is optimal for the species as a whole. Darwin said (1883, p. 259: Fisher cites this as p. 399, and the year 1871 by implication): 'I formerly thought that when a tendency to produce the two sexes in equal numbers was advantageous to the species, it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution for the future.' Kalmus & Smith (1960) argued that numerical equality of the sexes at maturity is advantageous for a given population because the effective genetic size of the population is then at a maximum, thus minimizing the inbreeding, and at the same time the chance of the meeting of two members of opposite sex is maximized. Campos Rosado & Robertson (1966) conclude more generally that a consequence of natural selection would be a maximization of the number of *successful* encounters between males and females; on a simple model, this would mean the evolution of a 1:1 ratio, but any realistic biological model would bring in complexities leading to a departure from this ratio.

The latter two authors stress that the nature of the gene action is critical. How, in fact, can sex ratio be altered genetically without provoking immediate counter-selection? The most economical model that could be imagined would be by differential production or differential fertility of X- and Y-bearing spermatozoa, which should leave the total numbers of offspring born unaltered. But the mechanism has no experimental justification. Another biologically efficient method would be by an accumulation of genes that cause a phenotypic sex reversal of a proportion of one of the sexes of offspring. This would have the complication that the mating of an XY female (sex reversed male) to a normal XY male would give distorted sex ratios, as well as the disadvantage of giving one class of progeny (YY) that would be inviable in higher vertebrates (though not in fish and Amphibia). Further, in higher vertebrates, there is growing evidence that XX gonadal cells cannot differentiate into functional spermatozoa, nor can XY cells give eggs. Another mechanism that certainly does affect the sex ratio is mediated by differential embryonic mortality of the sexes. Here, however, counter-selection would be expected to operate, because a lost embryo is to some extent a loss of biological capital, and in a monotocous species a whole period of the effective breeding life of the mother would also be lost. Genetic change in sex ratio mediated by differential mortality from birth onwards would

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

involve a greater loss of biological capital and would be expected to invite correspondingly greater counter-selection.

So, in summary, the Darwin–Fisher 'automatic 1:1 tendency' is an anticipated necessary consequence of natural selection, and Fisher's concept of parental expenditure can account for deviations from equality and for changes in sex ratio during development. There are also arguments that a 1:1 ratio is biologically *advantageous* for a species but the exact mode of gene action deserves continued study.

Which is the heterogametic sex in the species of vertebrates?

In the XX/XY sex chromosome mechanism of man and other animals, the female (XX) is the homogametic sex because it produces only one kind of gamete (X); the male (XY) is heterogametic because of its two kinds of gamete (X and Y). In the axolotl and other animals the situation is reversed, and different letters of the alphabet are used to remind us of the situation; the female (ZW) is the heterogametic sex, while the male (ZZ) is homogametic. In order to refer simultaneously to both mechanisms, I will call the Y and W chromosomes the 'odd' chromosomes, the X and the Z 'even' chromosomes. My intention now is to set up a check-list of the identity of the heterogametic sex in the vertebrates, omitting instances of multiple sex chromosomes, and omitting a good deal of cytogenetic detail for which I refer you to my 1964 review.

Ideally, we want for each species an unequivocal demonstration of visibly different sex chromosomes in one sex, and of identical sex chromosomes in the other sex. This is the most direct form of evidence possible. But not all species have been examined, not all are technically suitable for cytological analysis, and small differences would be obscured by the limits of resolution of the microscope. Fortunately, there are two other direct methods of identifying the heterogametic sex. One is by study of sex-linked inheritance, the other by study of the sex of progeny born to hermaphrodite or sex-reversed parents. Both these additional methods give patterns of inheritance from which virtually mandatory inferences can be made about the identity of the heterogametic sex.

When none of these reliable direct methods is available, we can still obtain preliminary indications from several indirect forms of evidence. One of these is the rule of Haldane (1922): 'When in the F_1 offspring of two animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex': few exceptions to this rule are known. Other indirect methods include arguments from the sex of triploids, from the sex of parthenogenetic and androgenetic offspring, and from what I have called Jost's first and second rules concerned with sex-reversal by hormone administration and with the 'anhormonal' sex that develops after early castration. These forms of direct and indirect evidence, for all vertebrate species to which they could be applied, are summarized in table 1. In fish, the heterogametic sex is sometimes the male, sometimes the female, according to the species or even the race. In urodele Amphibia, set out in three taxonomic groups, most species show male heterogamety with the definite exceptions of *Pleurodeles* and the axolotl. In anuran Amphibia, the situation is confused for the species of *Rana* but there is no doubt that the female is the heterogametic sex in *Xenopus*: reliable cytological evidence is lacking in Anura (Volpe & Gebhardt 1968). In higher vertebrates the position is quite clear; in the few reptiles studied and in birds the female is the heterogametic sex; in mammals it is the male.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 1. THE HETEROGAMETIC SEX IN VERTEBRATES

		direct evidence			*indirect evidence*			
organism	assessment of heterogametic sex	chromosomes	sex linkage	breeding from sex-reversed or hermaphrodite animals	response to castration or sex-reversing hormones	hybrids	triploidy	androgenesis
fish								
Gasterosteus 1 sp.	♀	—	—	—	—	—	♀	—
Lebistes races	♂*, ♀*	—	♂, ♀	♂, —	—	—	—	—
Mogrunda 1 sp.	♂	♂	—	—	—	—	—	—
Oryzias races	♂*, ♀*	—	♂, ♀	—	—	—	—	—
Xiphophorus races	♂*, ♀*	—	♂, ♀	—, ♀	—	—	—	—
urodele Amphibia								
Hynobius 2 spp.	♂	—	—	—	—	♂	—	—
Triturus 1 sp.	♂	—	—	—	—	♂	♂	—
Triturus 3 spp.	♂	—	—	—	—	♂	—	—
Triturus 1 sp.	♂	—	—	—	—	—	—	♂
Triturus 1 sp.	♂	—	—	—	—	—	♂	—
Oedipina 3 spp.	♂*[2]	♂	—	—	—	—	—	—
Thorius 3 spp.	♂*[2]	♂	—	—	—	—	—	—
Chiropterotriton 1 spp.	♂*[2]	♂	—	—	—	—	—	—
Pleurodeles 1 sp.	♀*	—	—	♀	—	—	—	—
Axolotl 1 sp.	♀*	—	—	♀	—	—	♀?	—
anuran Amphibia								
Xenopus 1 sp.	♀*	♀[1]	—	♀	—	—	—	—
Bufo 1 sp.	♀?	—	—	♀?	—	—	♀?	—
Rana 1 sp.	♀?	—	—	—	—	♀	—	—
Rana 2 spp.	♂?	—	—	♂	—	♀	—	—
Rana 1 sp.	♂?	—	—	—	—	♂	♀?	—
reptiles	♀*	♀	—	—	♀	—	—	—
birds	♀*	♀	♀	—	♀	♀	—	—
higher mammals	♂*	♂	♂	—	♂	♂	—	—

Definite assessments are marked*. Direct evidence is preferred when in conflict with indirect. (1) Weiler & Ohno (1962), but no sex chromosomes seen by Mikamo & Witschi (1966). (2) Unpublished results by kind permission of Dr J. Kezer. The table is modified from Beatty (1964), where the remaining references are listed: the former chromosomal evidence for a *Triturus* species has been deleted as non-critical.

Of two minor points emerging from the check-list, the first is that no one seems to have put forward any causal reason based on selective advantage as to why certain species have male and others have female heterogamety. The second point concerns the factual record. The literature is permeated with an older idea that the urodele Amphibia (especially the *Triturus* species) have female heterogamety, and that the anuran Amphibia have male heterogamety. I think these ideas must be abandoned. It seems more likely that the heterogametic sex is usually the male in urodeles (especially in *Triturus*), while in the Anura the only firm result is female heterogamety in one species.

But the main purpose of constructing the check-list was to provide a basis for the next question.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

What is the role of sex chromosomes and autosomes in determining gonadal sex of vertebrates?

Simple knowledge of the identity of the homogametic and heterogametic sexes is a step forward, and an essential step, but it tells us very little about the play and interplay of the sex chromosomes and autosomes in determining the sex of the gonad. For example, if we know that XX/XY mechanism exists, one could form the theory that the two X chromosomes of the female body determine a female gonad, and the single X chromosome of the male determines a male gonad. Or, one could imagine that the Y chromosome determines the male gonad, and absence of Y determines a female gonad. Other theories could be developed. In fact, there is no single mechanism applicable to all animals. In *Drosophila* (Bridges 1939) the sex of the gonad is determined by a balance between the number of autosomes (male-determining) and the number of X chromosomes (female-determining) the Y being a dummy from the point of view of gonadal sex determination. In mammals, on the other hand, the Y chromosome is not sexually a dummy; it has a strong male-determining effect (Welshons & Russell 1959).

Therefore, to disentangle in vertebrates the separate effects on gonadal sex of the 'even' chromosomes (X or Z) the 'odd' chromosomes (Y or W) and the autosomes, the obvious step taken by many authors long ago is to follow the classical principles of experimentation by varying independently of one another the factors that are being studied. This means, simply, that one must study animals with abnormal chromosome complements, either by taking advantage of naturally occurring deviants, or else by bringing them into existence by special laboratory techniques. I therefore went through the available literature on chromosomal deviants with the intention of recording two things for as many vertebrates as possible: the chromosome constitution, as a cause, and the gonadal sex, as an effect. Chromosomal mosaics were omitted as being insufficiently documented at that time. Again, I will refer you to my 1964 review for cytogenetic details, and present only a check-list of results in table 2.

An obvious harmony exists in table 2. Apart from the three hermaphrodites, one of them a 'hermaphrodite in time', it will be seen that *in each species* all individuals with an odd chromosome are of one and the same gonadal sex, the actual sex being characteristic of the species; all individuals without an 'odd' chromosome are of the opposite sex. There is no such harmony relating gonadal sex to the number of 'even' chromosomes, or to the number of autosomes, or to the ratio between these two numbers. This may be expressed in the form of the following rule, remembering that chromosomal mosaics have been excluded. If we take from any one vertebrate species individuals with a known chromosomal constitution and a known gonadal sex, then if at least one 'odd' chromosome is present, these individuals will all have one and the same gonadal sex (except for occasional hermaphrodites). I interpret this in terms of cause and effect. It seems that the 'odd' chromosome has a powerful and universal effect in determining gonadal sex. Let me be clear; the point is that the 'odd' chromosome *determines* gonadal sex; I do not and cannot explain why it should be the male sex in some species and the female in others. What determines the gonadal sex that develops in the absence of 'odd' chromosomes? Instinctively, one thinks that the 'even' chromosomes should play a role. But there is little evidence in vertebrates that they rather than the autosomes are implicated. I am grateful to my colleague Dr D. M. Woolley for leading me to revise the way in which these matters were set out in my 1964 review; in particular, by omitting an unnecessary form of words, the 'heterogametic (or homogametic) sex type'.

There is nothing original on my part in the idea of assessing the identity of the heterogametic sex and of relating chromosomal deviants to gonadal sex. In particular, I would like to emphasize that my conclusions do not antedate in any way the important finding of the male-determining effect of the mammalian Y chromosome, made by members of this Discussion and their colleagues. My only points of attempted originality are to appraise the vertebrate literature as a whole, and to assert the sexual paramountcy of the 'odd' chromosome, Y or W, for vertebrates in general.

TABLE 2. CHROMOSOME CONSTITUTION AND GONADAL SEX IN VERTEBRATES, EXCLUDING CHROMOSOMAL MOSAICS. MODIFIED FROM BEATTY (1964): ADDITIONAL REFERENCES IN FOOTNOTE

		mammals			amphibia			amphibia			birds[2]	fish
sex chromosomes		man	mouse	cat, sheep[1]	*Triturus alpestris*	*T. pyrrhogaster*	sex chromosomes	*Amby-stoma*	*Pleuro-deles waltlii*	*Xenopus laevis*	chick	*Gaster-osteus*
						Euploids						
X	+1A	—	—	—	—	♀	Z	+1A ♂	—	—	—	—
XX	+2A	♀	♀	♀	♀	♀	ZZ	+2A ♂	♂	♂	♂	♂
XXX	+3A	♀[3]	—	—	♀	♀	ZZZ	+3A ♂	♂	♂	—	♂
XXXX	+4A	—	—	—	—	♀	ZZZZ	+4A ♂	—	♂	—	—
XXXXX	+5A	—	—	—	—	—	ZZZZZ	+5A ♂	—	—	—	—
XY	+2A	♂	♂	♂	♂	♂	ZW	+2A ♀	♀	♀	♀	♀
XYY	+3A	—	—	—	—	—	ZWW	+3A ♀	♀	♀	—	♀
XXY	+3A	♂[3]	—	—	♂[4]	♂	ZZW	+3A ♀	—	—	♀	—
XXYY	+4A	—	—	—	—	♂	ZZWW	+4A ♀	—	♀	—	—
XXXY	+4A	—	—	—	—	—	ZZZW	+4A ♀	—	—	—	—
XXXYY	+5A	—	—	—	—	—	ZZZWW	+5A ♀	—	—	—	—
XXXXY	+5A	—	—	—	—	♂	ZZZZW	+5A —	—	—	—	—
YY	+2A	—	—	—	—	—	WW	+2A ♀	—	♀[5]	—	—
YYY	+3A	—	—	—	—	—	WWW	+3A ♀?	—	—	—	—
						Aneuploids						
X	+2A	♀	♀	—	—	—						
XXX	+2A	♀	—	—	—	—						
XXXX	+2A	♀	—	—	—	—						
XXXXX	+2A	♀[6]	—	—	—	—						
XXY	+2A	♂	♂	♂	—	—						
XXXY	+2A	♂	—	—	—	—						
XXXXY	+2A	♂	—	—	—	—						
XYY	+2A	♂[7]	♂[8]	—	—	—						
XXYY	+2A	♂	—	—	—	—						

no data

(1) Bruere *et al.* (1969). (2) ZW:ZZ mechanism assumed. (3) Edwards *et al.* (1967). (4) Eventually male, initially female. (5) Mikamo & Witschi (1966). (6) Miller & Warburton (1968). (7) Jacobs *et al.* (1965). (8) Cattanach & Pollard (1969).

WHAT ARE THE SEX-DETERMINING FACTORS WITHIN CHROMOSOMES?

The discovery that sex was determined genetically used whole individuals as units of observation. The discovery of the cytological basis of sex determination employed whole chromosomes as observational units, and intra-chromosomal units were used as soon as it became evident that sex is determined chromosomally by the inheritance of the differential or non-pairing segments of chromosomes. Logically, the next step is to use still smaller units of observation—blocks of genes, single genes and so on. In vertebrates, I have taken a tentative step away from these smaller units by suggesting (Beatty 1967) that just as mankind is human because of the total gene complex, and we do not go looking for specific genes causing humanness, it might also be that a

woman is female because of her total gene complex, and not because of a few specific genes causing femaleness. The role of intra-chromosomal entities in sex determination will be considered in more detail by other speakers, one of whom has reviewed the subject (Mittwoch 1967) and considers whether the ultimate genetic sex-determining units are perhaps aggregates of genes, or heterochromatin, or even whole chromosomes; she also recalls that specific sex-determining genes cannot be demonstrated in *Drosophila*, except for a few special mutations.

Can sex ratio at conception be controlled?

My final question deals with the more genetical aspects of the possibility of controlling sex ratio at conception. Obviously, we can and do brutalize the sex ratio in either direction by killing or not officiously keeping alive the members of one sex in plants and animals—even in man. We can predict the sex of unborn children by nuclear sexing of cells from amniotic fluid (Sachs, Serr & Danon 1956); if we were to abort a foetus of a given sex, which would sometimes be ethical if the foetus was known to be at genetic risk, we would be affecting the sex ratio at birth. Gardner & Edwards (1968) were able to sex living rabbit blastocysts, and therefore by transfer of eggs a given host could be made to bear offspring of a predetermined sex. But is there a possibility of controlling sex ratio at the moment of conception by causing more X-bearing (female-determining) or else more Y-bearing (male-determining) spermatozoa to fertilize an egg? Could we physically separate the two classes of spermatozoa, or differentially destroy or inactivate one class? If this were possible, the normal process of procreation would not otherwise be interfered with, and no individuals would be sacrificed, even as embryos.

The possibility of ever being able to control sex ratio in this way hinges on the reality or otherwise of a more general phenomenon that I will call a 'haploid effect'. A haploid effect would be a specific effect of the haploid genetic content of a spermatozoon on its own phenotype, for example on its size, motility, fertility and so on. A particular example of this would be a differential effect of X and Y chromosomes on the spermatozoa bearing them. If we are ever going to be able to separate X- and Y-bearing spermatozoa there will *have* to be a haploid effect. Without it, no separation is possible.

There are formidable reasons for supposing that a haploid effect both cannot occur and does not occur. For the particular case of sex chromosomes, there is an *a priori* objection that Nature, having gone to all the trouble of arranging for a certain proportionality between the sexes is unlikely to have given different phenotypes to X- and Y-bearing spermatozoa, with the danger that fortuitous environmental fluctuations might affect the one kind of spermatozoa rather than the other and thus give uncontrolled fluctuations in the ratio. Rather, one would expect that natural selection would avoid such disturbances by producing physically identical X- and Y-bearing spermatozoa.

But returning to the haploid effect in general; it is generally supposed that the nucleus of a spermatozoon is in a semi-crystalline state, with gene action in abeyance, and no haploid effect mediated by gene action would be possible. Although protein synthesis has been reported in spermatozoa it does not seem to be mediated by RNA elaborated by the specific genotype of the spermatozoon. Monesi (1965) has evidence in the rat that messenger RNA is not synthesized by the sex chromosomes during male meiosis. Again, therefore, there is no basis for a haploid effect.

There is also evidence that a haploid effect *does not* occur. Many experiments with gross

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

abnormalities in the number or structure of chromosomes show that all the spermatozoa of a male, irrespective of their genetic content, seem to have the same fertility. Then again, various attempts have been made to see whether a male heterozygous for some Mendelian factor, such as the blood groups, produces spermatozoa with two different phenotypes, those with the one antigen and those with the other. I think Dr Edwards and I would agree that there has been no certain demonstration of a haploid effect in this kind of work. There have also been empirical attempts to separate or differentially destroy X and Y spermatozoa, but there seems to be no confirmed success. In spite of earlier claims, there seems also to be no real evidence that X- and Y-bearing spermatozoa differ in size.

Therefore, it is entirely possible that control of the sex ratio by this means is no more than a pipe-dream, that it can never be realized, and I think that for elementary teaching purposes one would go no further than stating this sombre conclusion. But, when a thing is said to be impossible, research does not automatically stop all over the world. On the contrary, it may even be stimulated. And, after all, X and Y chromosomes are physically different, and X- and Y-bearing spermatozoa cannot therefore have absolutely identical phenotypes.

The most positive stimulus for further research comes from a single and apparently well-established instance of a real haploid effect in mammals, the crucial experiment being that of Braden (1958). The work has been fully confirmed by Yanagisawa, Dunn & Bennett (1961). Braden studied the 'tailless' locus in the mouse, a locus that can be occupied by a number of alleles such as T, t and the wild-type allele $+$, that affect the length of the mouse's tail. It had been known for many years that segregation at this locus is normal through the female parent, but abnormal through the male parent. Using normal mating procedure, he mated heterozygous males (Tt) to females that for present purposes I will symbolize as $++$. He obtained the two types of progeny $T+$ and $t+$ in ratio 23:77. This is not a 1:1 Mendelian ratio, but that is not relevant for the present purposes; let us think of it simply as a control ratio. He then carried out delayed matings, late in oestrus, and found that the ratio had changed to 40:60. Evidently, the transmission of T and t to offspring via the spermatozoa depended on the experimental conditions.

Now the experimental change in the segregation ratio had nothing to do with changed production of the numbers of T- and t-bearing spermatozoa within males, because the spermatozoa were already in existence in the males. It was also known that the changed ratio was not due to differential embryonic mortality. The only possibility was that T and t spermatozoa must respond in different ways to the experimental challenge; they must have different phenotypes in terms of this response. A genetic content of T must give one phenotype, a genetic content of t another phenotype. This seems, therefore to be a unique case of a haploid effect in an animal. There seems to be one crack in the dogma that a haploid effect cannot occur and that separation of spermatozoa according to their genetic content would be for ever impossible.

I trust that I have not told my colleagues too much of what they already know. This is an inherent risk of the first contribution to a Discussion. I am glad to see in the audience one of the fathers of sex-determination, Professor F. A. E. Crew, and may I say how pleased I am to have a former but long-ago independent pupil, Dr R. G. Edwards as Chairman? The quotation from Fisher (1930) is by permission of the Clarendon Press, Oxford.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Discussion on paper by R. A. Beatty, p. 3

A. McLaren (*Institute of Animal Genetics, Edinburgh University*): Beatty's exposition of Fisher's selective theory rests on the assumption of a monogamous mating system. In a polygamous association, i.e. where males have harems of females and therefore only those individuals who are successful in acquiring a harem will leave progeny, a different situation exists. Here individuals of above average genetic worth will contribute more to future generations if they also carry genetic factors favouring a high male sex ratio. On the other hand, inferior individuals will do better if most of their progeny are females, because in any such population every female is likely to contribute to some extent to future generations. Since the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

superior males are going to contribute a lot to future generations and the inferior males are going to contribute much less, this could lead to a high male sex ratio. Now this seems paradoxical because biologically a low male sex ratio would seem more appropriate for the harem system. Is there any data on the actual sex ratio among polygamous species?

A. Jost (*Laboratoire de Physiologie Comparée, Paris*): In experiments concerning sex reversal under the influence of sex hormones there is a general tendency to find the homogametic sex very sensitive to the hormone of the heterogametic sex. But there are exceptions such as the opossum and some Amphibians (*Triturus* according to Dr Beatty's table); could he comment on such cases?

R. A. Beatty: Amphibia are a little difficult to score because of spontaneous sex reversal. The endocrinological method of determining and altering the differentiation of sex is very important, but for present purposes I think of it as an indirect and not infallible method of establishing heterogametic sex and so I am not too concerned if there are exceptions.

R. G. Edwards (*Physiological Laboratory, Cambridge*): In *Drosophila* there are genes which cause distortions in the sex ratio. Obviously, some of these will be sex-linked lethals, but there are others which affect the proportion of male offspring in a manner similar to that found by Braden for the ratio of T to t offspring. Is there any evidence to suggest that the observations on mice can apply to *Drosophila* or other species?

R. A. Beatty: The work in mice could have far-reaching implications. There is a similar phenomenon in *Drosophila* which is explained in a totally different way not involving haploid effects. The explanation devised for *Drosophila* was that two types of egg exist, one which is physiologically abnormal and the other physiologically normal. Certain gene segregations are postulated to be preferential during meiosis in heterozygous males: more spermatozoa carrying one of the alleles are produced and fertilize more eggs than those carrying the other allele. Thus, distorted ratios result. The results obtained by A. W. H. Braden could also be explained in this way, but I am a little critical of the *Drosophila* interpretation for reasons to be published elsewhere.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES