MULTIPLE ALLELES AND SEX DETERMINATION IN HABROBRACON¹

C. H. BOSTIAN

North Carolina State College, Raleigh, North Carolina

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INTRODUCTION

THE occurrence of biparental males in the wasp, *Habrobracon juglandis* (Ashmead) demonstrates that sex is not determined simply by the fertilized or unfertilized condition of the eggs. When females having any recessive character are mated to unrelated males with the dominant allele, the male offspring exhibit the recessive trait, the female offspring the dominant. If the parents are related, some males appear which, unlike their normal haploid brothers, show the dominant trait. These biparental males have 20 chromosomes like their sisters, in contrast to the 10 of their haploid brothers.

The first satisfactory explanation for the determination of sex in this wasp was suggested by WHITING (1933a), who proposed that femaleness is caused by heterozygosity for an allelic pair of sex factors or chromosomes, X and Y, along with two sets of autosomes. Thus the chromosomes of a female are $2A+X+Y$. Haploid males are $A+X$ or $A+Y$. Diploid males are $2A+XX$ or $2A+YY$. Two lines of evidence support this explanation.

First, the genitalia of bilateral mosaics, produced by virgin mothers, frequently show feminization if the line of demarcation between the two types of tissue passes near the genitalia. This feminization is assumed to be caused by a substance produced by the Y factor which diffuses into the X tissue. These mosaic males result from the simultaneous cleavage of two haploid nuclei, genetically different.

Second, the locus for fused (*fu*) antennae was found to be linked with sex-determining factors (WHITING 1933b). A certain fused male, designated *fu.X*, was crossed with two of his daughters. This mating yielded 14 type daughters and one fused daughter in contrast to the 1:1 ratio which might have been expected. To show the linkage, the mating may be expressed as follows: $Fu.Y/fu.X \times fu.X \times fu.X$, producing as offspring 14 type females, $Fu.Y/fu.X$, and one fused female, a crossover, $fu.Y/fu.X$. Subsequent crosses gave about ten percent crossing over between *fu* and the sex factors. Experiments reported by BOSTIAN (1935a) were made so that haploid and diploid males could be distinguished by their eye color. Orange-eyed females heterozygous for fused were crossed with closely re-

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lated black-eyed fused males. Results are recorded in table 1. In Group 1 the percentage of *fu* among females is 82.7, while in Group 2 the percentage is 10.9. Crossovers are more frequent when represented by type females, as in Group 1, than when represented by *fu* females, as in Group 2, because of viability differences. Attention is called to the greater occurrence of type diploid males when fused females are in excess, as in Group 1, and the reverse condition as in Group 2.

This theory of sex determination had immediately one important difficulty facing it, the need for explaining the relative infrequency with which biparental males appear. If females are XY and haploid males either X or Y, half of all fertilized eggs should be either XX or YY, and hence produce diploid males. The fact is, however, that when unrelated wasps are crossed, no diploid males at all appear, and even in fraternities from related parents they may comprise less than one percent of the biparental offspring or they may range as high as 25 percent or more from crosses of certain stocks (BOSTIAN 1934).

TABLE 1
Sex linkage of fu as shown by diploid males and females.

FRATERNITIES		PARENTS		OFFSPRING					
				♀ ♀ BLACK EYES		DIPLOID ♂ ♂ BLACK EYES		HAPLOID ♂ ♂ ORANGE EYES	
Groups	No.	♀ ♀	♂ ♂	+	<i>fu</i>	+	<i>fu</i>	+	<i>fu</i>
1	13	<i>o/o</i> ; <i>Fu.X/fu.Y</i>	<i>O fu.X</i>	96	506	92	16	220	197
2	40	<i>o/o</i> ; <i>Fu.X/fu.Y</i>	<i>O fu.Y</i>	1575	193	41	230	577	510

This difficulty has been explained in part by differential mortality. The homeosyngamic male-determining combinations (XX and YY) are less viable than the heterosyngamic female-determining (XY) ones.

This reduced viability, however, cannot explain the total absence of biparental sons of outcrossed females, for in such fraternities egg hatchability and fecundity are high and the ratio of females to haploid males is practically doubled. It had been observed by several investigators that fewer offspring are obtained from mated females than from unmated, and from close crosses than from outcrosses. Counting of eggs with daily observations on their development (BOSTIAN 1935a) established a much reduced hatchability of eggs and increased mortality of larvae and pupae for matings which produced diploid males, as compared with matings which did not include any diploid males among the offspring.

Thus WHITING (1935) was led to suggest a theory of differential maturation of the egg. "The sex chromosomes of the sperm nucleus must deter-

mine that the innermost chromosome set shall contain the opposite type of sex chromosome." According to this theory all zygotes from outcrosses are thus female-producing, heterosyngamic, XY. When parents are related, more or less homeosyngamy occurs. Thus for any particular cross the production of diploid males depends first on the relative frequency of homeosyngamic combinations and second on the viability of these zygotes.

Subsequently SNELL (1935) suggested that selective fertilization was entirely unnecessary to explain the published data of WHITING, BOSTIAN, and others, if femaleness results from the heterozygosity of one or more of a number of sex factors, some of which are located on different chromosomes. Granting the operation of five or more such pairs of factors, the chance of unrelated parents giving diploid males in the F_1 becomes very slight.

According to SNELL, the locus fused is linked with one pair of these factors, and it may be expected that further linkage tests would reveal other sex factors on different chromosomes. Furthermore it would be possible for parents to be alike for the factors linked with fused, in which case sex determination would depend on other factors, and fused would not be sex-linked. The simplest kind of such a cross would be: $(Fu.X)(fu.X) WZ \text{ } \varnothing \text{ } \varnothing$ by $(fu.X) W \text{ } \sigma \text{ } \sigma$, and the resulting zygotes would yield type and fused females in a 1:1 ratio, and type and fused diploid males in a 1:1 ratio. Sex linkage of fused might also be masked in crosses of the following types: $(Fu.X/fu.X) WZ \text{ } \varnothing \text{ } \varnothing$ by $(fu.Y) W \text{ } \sigma \text{ } \sigma$ and $(Fu.X/fu.Y) ZZ \text{ } \varnothing \text{ } \varnothing$ by $(fu.X) W \text{ } \sigma \text{ } \sigma$. Here all the zygotes would be female-producing because heterozygous, XY or WZ, and of correspondingly good viability.

Before many crosses of Fu/fu to fu had been made, fraternities appeared where there was no apparent linkage of fused and XY. In these fraternities type and fused females were approximately equal in number, and, very significantly, there were no diploid males at all.

Two interpretations were suggested. WHITING (1935) proposed translocation with a break occurring between the loci of fused and sex. According to SNELL's hypothesis, sex was being determined by factors other than those linked with fused. More recently, on the basis of data presented by DORDICK (1937) and others (unpublished), WHITING has abandoned the hypothesis of differential maturation since "fused, shown to be sex-linked in close-crossed females, appears as if non-sex-linked if similar females are outcrossed." (Quoted from a letter by permission.) The subject up to this point has been briefly reviewed and discussed by BRIDGES (1939, pp. 54-58).

NEW DATA

In this paper data are reported which throw much doubt on the theory of multiple sex factors and indicate strongly that only one series of alleles

is concerned with sex determination. In table 2 are shown three groups of fraternities from orange-eyed females heterozygous for fused crossed with black-eyed fused males. Diploid males may therefore be separated by eye color from their haploid azygous brothers. More than half of these fraternities show independent segregation of fused and sex and such fraternities tend to be larger than those with linkage, indicating high viability of zygotes. They are therefore of the "outcross" type.

TABLE 2

*Sample matings showing absence of diploid males when fused segregates independently of sex factors.**

Group	Fraternities	OFFSPRING					
		FEMALES		DIPLOID MALES		AZYGOUS MALES	
		type	<i>fu</i>	type	<i>fu</i>	type	<i>fu</i>
1	467.3	2	12	8	3	4	5
	467.4	12	13			4	2
	467.5	1	12	11	2	4	8
2	468.8	28	22			7	4
	468.9	16	21			7	7
	468.10	11	12			9	8
3	473.1	8	51	18	10	17	19
	473.6	65	74			25	20
	473.8	46	47			24	22
	475.1	59	61			11	10
	475.6	14	70	42	9	29	19
	477.3	10	43	30	3	13	11

* Orange females heterozygous for fused (*o/o; Fu/fu*) were in all cases mated to black-eyed fused males (*O fu*). In Group 1, as also in Group 2, the females were related but not sisters and were mated to the same male. In Group 3, the females were full sisters, each mated to a different male.

On the basis of SNELL's hypothesis it appeared that it ought to be possible to develop a strain where the *fu* locus would be permanently free of linkage with the sex factors. It was also desirable to obtain more extensive data to confirm the preliminary conclusion that diploid males are never produced when *fu* fails to be sex-linked. Accordingly, for two years parents for crosses were selected from fraternities without apparent sex linkage of fused. This selection was carried through about 20 generations, and during the last 11 all crosses involved sibs. During these tests no exceptions were found to the rule that sex linkage of *fu* and the occurrence of diploid males is inseparable. Without one, the other does not occur. Nor was it possible to develop a strain breeding pure for independence of *fu* and sex.

This selection toward a strain permanently free of sex linkage was be-

gun and maintained in the following way. Mating 499.7 involved a brother-sister cross, $o/o; Fu/fu$ female to $O fu$ male, and the offspring were 64 type ♀♀ ($O/o; Fu/fu$), 63 fu ♀♀ ($O/o; fu/fu$), no diploid males, and 66 orange-eyed azygous males ($o Fu$ and $o fu$).

Seven of the F_1 type females, $O/o; Fu/fu$, were mated singly with $o fu$ brothers, and all matings gave independent segregation. Among the offspring, diploid males could not be distinguished in appearance from azygous males.

TABLE 3

Selection for strain showing independent segregation of fused and sex factors.

GENERATION	NUMBER OF MATINGS	MATINGS SHOWING INDEPENDENT SEGREGATION	MATINGS SHOWING SEX-LINKAGE OF <i>fu</i>
1	7	7	0
2	13	11	2
3	6	5	1
4	13	9	4
5	8	5	3
6	14	9	5
7	15	8	7
8	10	7	3
9	12	10	2
10	33	26	7
11	13	10	3

Continuing the selection, two $o/o; Fu/fu$ females from one of the seven matings just mentioned were crossed to an $O fu$ brother. Both crosses gave independent segregation and no diploid males.

In this way selection was continued for a number of generations. Alternate generations involved as parents orange-eyed females and black-eyed males and thus the male offspring could be separated into diploid and azygous classes. In every generation the female parents were heterozygous for *fu*, and the male parents were *fu*, so that a measure of sex-linkage was always present.

In table 3 is presented a summary of this selection, as carried out from the time brother-sister matings exclusively were made. Diploid males were not separable from azygous ones in the odd-numbered generations.

DISCUSSION

The failure to develop a strain free of linkage of *fu* and the sex factors seems to be thoroughly established, and along with the consistent absence of diploid males when *fu* is independent of sex, necessitates a hypothesis different from the multiple factor scheme of SNELL.

As pointed out earlier, SNELL's hypothesis would allow the controlling factors to be a pair independent of *fu*, but does not permit any explanation for the failure of diploid males to appear after inbreeding. Nor does it

account for the seeming impossibility of developing a strain permanently free of sex linkage of *fu*.

The conclusion seems warranted that any theory of sex determination must recognize the fundamental significance of the relation of sex-linkage of *fu* and the production of diploid males. This condition and all other pertinent data as well are satisfied by WHITING's suggestion (1939 and personal correspondence) that multiple alleles are involved, x^a , x^b , x^c , x^d , etc., instead of a single pair of factors as XY. Femaleness may be considered as due to heterozygosity for any two alleles, and diploid maleness to homozygosity for any one.

When this hypothesis is applied to the experiment reported above, the seven matings of generation one (see table 3), can be indicated in the following way:

	$x^a.Fu/x^b.fu$	♀ ♀	by	$x^c.fu$	♂ ♂
Haploid offspring:	non-crossovers:	$x^a.Fu$,	type male	and	$x^b.fu$, fused male
	crossovers:	$x^b.Fu$,	type male	and	$x^a.fu$, fused male
Diploid offspring:	non-crossovers:	$x^a.Fu/x^c.fu$,	type female		
		$x^b.fu/x^c.fu$,	fused female		
	crossovers:	$x^a.fu/x^c.fu$,	fused female		
		$x^b.Fu/x^c.fu$,	type female		

Here all diploids are heterozygous for the alleles, being x^a/x^c or x^b/x^c , and hence all are females.

In the second generation, eleven matings showed segregation, and two showed linkage. Here the most likely crosses of type females to their fused brothers would be $x^a.Fu/x^c.fu$ by $x^b.fu$, and their offspring would not include diploid males. The matings producing diploid males and showing sex-linkage were of two possible kinds: $x^a.Fu/x^c.fu$ by $x^a.fu$ or $x^b.Fu/x^c.fu$ by $x^b.fu$. In the first case the female is a non-crossover and the male a crossover, and in the second the female is a crossover and the male a non-crossover.

In the third generation, five of the six matings resulted in independent segregation, as would be expected from crossing the non-crossover females, $x^a.Fu/x^b.fu$, to their non-crossover brothers, $x^c.fu$. The non-fused daughters would be approximately 90 percent $x^a.Fu/x^c.fu$ and 10 percent $x^b.Fu/x^c.fu$, and the *fu* sons would be $x^b.fu$ and $x^a.fu$ in the same ratio.

In the fourth generation, nine matings gave independent segregation. They were probably the $x^a.Fu/x^c.fu$ females by the $x^b.fu$ males. The four matings giving linkage could have been $x^b.Fu/x^c.fu$ females by $x^b.fu$ males or $x^a.Fu/x^c.fu$ females by $x^a.fu$ males.

In the next seven generations fraternities of both types occurred in each series of matings, with those showing free segregation always in the majority. With sex determination depending on multiple alleles linked with *fu*, the impossibility of developing a strain where all crosses give independent segregation is apparent. By selecting parents from a fraternity which gave

segregation three different alleles become involved, and all crosses will show segregation except for the crossovers which occur.

Further critical tests of this scheme of multiple alleles are possible. Crosses of fathers to daughters, and mothers to sons, should yield diploid males even though the original parents were entirely unrelated. Unpublished data of PROFESSOR P. W. WHITING show this to be true.

That quite a large number of these alleles may exist is suggested by the consistent failure of crosses of unrelated stocks to produce diploid males. Inbreeding following such outcrosses has always resulted in diploid males, however, with increasing frequency of occurrence with more inbreeding. In fact, when very closely related stocks are crossed, it is usually true that one hundred percent of the fraternities have diploid males. The failure of the diploid males to equal the females is due to greatly reduced viability of zygotes destined to be diploid males.

This scheme of multiple alleles with heterozygosity producing femaleness and homozygosity resulting in diploid maleness is somewhat analogous to the inheritance of self sterility in *Nicotiana* (EAST and YARNELL 1932). In the latter case evidence for as many as fifteen alleles has been found.

SUMMARY

Failure to establish a strain free of linkage of *fu* and the sex factors and the consistent lack of any diploid males when linkage is not present make very improbable SNELL's hypothesis of multiple factors for sex determination in *Habrobracon*.

A scheme of multiple alleles, as recently suggested by WHITING, (1939) with femaleness depending on heterozygosity and diploid maleness on homozygosity, fits the data and evidence known at the present time.

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