MOSAICISM IN *HABROBRACON JUGLANDIS* ASSOCIATED WITH THE EBONY LOCUS¹

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AMONG species of insects that have been used for genetic studies, there occasionally appear adults which are composed of two or more different genomes. In *Drosophila melanogaster*, where most of these mosaics were of the gynandromorph type, it has been shown that elimination of an X chromosome during early cleavage was involved (MORGAN and BRIDGES 1919; PATTERSON 1931). In a few instances, fertilization involving two egg pronuclei and two sperm was suggested (BRIDGES 1925). In *D. pseudoobscura*, CREW and LAMY (1938) have obtained unisexual mosaics of the haplo-diploid type and have suggested that the sperm pronucleus divides before union with the egg pronucleus, that one of these cleavage nuclei unites with the egg pronucleus to form a diploid nucleus while the other sperm nucleus continues to divide and to give rise to haploid tissue.

Among insect species with haploid parthenogenesis, the gynandromorph types contain diploid female structures arising from the union of an egg and a sperm pronucleus, and haploid male structures arising from an accessory pronucleus. In *Habrobracon juglandis* the accessory pronuclei may be of maternal or paternal origin (P. W. WHITING 1932; A. R. WHITING 1961). In the honey bee, *Apis mellifera*, dispermy is involved (ROTHENBUHLER, GOWEN and PARKS 1952). In *Habrobracon*, haploid mosaic males derived from unmated heterozygous females occur and have been shown to consist of genetic material from two and sometimes three genetically different pronuclei (A. R. WHITING 1961; VON BORSTEL 1957). In haploid organisms, a model based on chromosome elimination does not apply since such loss would be lethal.

The fact that mosaicism appears more frequently in some stocks than in others and sometimes in association with a single mutant gene may indicate that the production of mosaics is under genetic control. There may be a relation between specific types of mosaics and specific mutants, and thus the particular developmental events that precede the union of egg and sperm nuclei may each be under control of a gene. If this is true, then the process of fertilization can be studied from the standpoint of developmental genetics. The present paper shows that events leading to fertilization are under genetic control. This conclusion is based on a study of mosaics in *Habrobracon juglandis* that are associated with the *ebony* locus.

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MATERIALS AND METHODS

In the parasitic wasp, *Habrobracon juglandis* (Ashmead), haploid males are produced from unfertilized eggs, and diploids, both male and female, from fertilized eggs. The haploid males are thus of gynogenetic (maternal) origin while the diploids are of zygotenetic (biparental) origin. Sex is determined by a series of multiple alleles, designated *xa*, *xb*, *xc*, etc. Haploid males bear any one of these alleles; diploid females are heterozygous; diploid males are homozygous (P. W. WHITING 1943). In addition to gynogenetic and zygotenetic progeny, mosaics are produced. The scheme for detection of unusual types is shown in a cross involving black body color females with honey body color males (Table 1). Black and honey are not alleles and both are recessive to the wild type body color. Listed are the phenotypes of the progeny and their possible parental origin. The symbol (G) represents a female haploid nucleus; (A) represents a male haploid nucleus; (Z) represents the union of the two nuclei. In *Habrobracon* as in *Drosophila* and *Apis* the principle of cell autonomy generally holds, so that the genetic constitution of each part of the adult can be recognized.

In addition, sex mosaicism in *Habrobracon* can be recognized by differences in external anatomy. Males and females differ not only in their genitalia, but also in antennal length and in the size of the sternites on the ventral surface of the abdomen. Characteristic mating and egg laying behavior enable one to distinguish gynandromorphs that are "psychic" males from those that are "psychic" females. Abnormalities in the internal anatomy of the reproductive organs are also diagnostic of gynandromorphs.

In *Habrobracon*, mature eggs are stored in the uterus and are arrested in first meiotic metaphase. Sperm are stored in the spermatheca. In oviposition, the eggs are squeezed through the ovipositor. The meiotic arrest is then broken and meiosis is completed within 30 minutes at 25°C (SPEICHER 1936). Eggs laid may be either fertilized or unfertilized. Among fertilized eggs, about one per cent are dispermic (SPEICHER 1936).

The recessive mutant *ebony* produces a black body color phenotype. In addition, it was observed that gynandromorphs appeared frequently in the *ebony* stock. Crosses were made involving *ebony* and other mutant genes in order to determine its relation to the production of mosaics and the types of mosaics produced. These crosses are considered in each experiment.

RESULTS

In *Habrobracon* there are four recessive mutants that produce black body color and are alike in phenotype. *Black* and *black*¹ are alleles; *ebony* and *ebony*¹ are alleles. The incidence of mosaics was determined in these stocks as well as for the wild type stock #1. The progeny obtained from these crosses were classified as gynogenetic males, zygotenetic females and mosaics (Table 2). The

TABLE 1
Black body color females × honey body color males

Phenotype of progeny	Presumed nuclear origin
black ♂	gynogenetic (G)
type ♀ or ♂	zygotenetic (Z)
honey ♂	androgenetic (A)
black + honey ♂ (mosaic)	gyno. + andro. (G + A)
type + black (mosaic)	zygo. + gyno. (Z + G)
type + honey (mosaic)	zygo. + andro. (Z + A)
type + black + honey (mosaic)	zygo. + gyno. + andro. (Z + G + A)
type ♀ + type ♂ (mosaic)	zygo. + zygo. (Z + Z)

TABLE 2

Incidence of mosaicism for the ebony mutants in Habrobracon

Cross ($\text{♀} \times \text{♂}$)	Gynogenetic $\text{♂} \text{♂}$	Zygogenetic $\text{♀} \text{♀}$	Mosaics	M/♀
$+/+ \times +$ (#1 stock)	2,093	675	0	.000
$e/e \times e$	837	436	30	.069
$e/e \times +$	426	136	12	.088
$+/+ \times e$	696	1,301	1	.001
$e^1/e^1 \times e^1$	859	556	37	.067
$+/+ \times e^1$	774	916	0	.000
$e^1/e^1 \times +$	908	752	29	.039
$bl/bl \text{ o/o} \times bl \text{ o}$	683	415	1	.002
$bl^1/bl^1 \text{ o}^i/\text{o}^i \times bl^1 \text{ o}^i$	556	563	0	.000
e/e (unmated)	1,023	0	0	.000

incidence of mosaicism was expressed as mosaics per female since the gynogenetic male frequency may vary due to the sperm supply. From crosses involving the wild type, the *black*, *orange-eyed*, and the *black*¹, *ivory-eyed* stocks, 1 mosaic was obtained among 1653 female progeny. For crosses involving the *ebony* and *ebony*¹ mutants ($e/e \times e$, $e^1/e^1 \times e^1$) the frequency of mosaics among the progeny was 0.069 and 0.067, respectively. For crosses in which the mothers were e/e or e^1/e^1 , the frequency of mosaicism was high, while in crosses in which the fathers were e or e^1 and the mothers were non-*ebony*, the frequency was the same as that for the non-*ebony* stocks. High mosaic production therefore depends upon the presence of the *ebony* gene in the mother. Unmated females produced no mosaics. Indeed, they could not be detected were they to occur in the homozygous stock.

Crosses were made to determine the dominance relations of the *ebony* mutant. The indentation of some crosses in Table 3 is meant to show that the females were progeny of the preceding cross. The data show that mated heterozygous mothers produced the same frequency of mosaics as those mated females that

TABLE 3

Incidence of mosaics for the ebony mutant

Cross ($\text{♀} \times \text{♂}$)	♀ ♀	Mosaics	M/♀
$e/e \times e$	708	40	.056
$+/+ \times +$ (#1 stock)	677	0	.000
$e/e \times +$	355	18	.051
$e/+ \times e$	170	0	.000
$e/+ \times +$	300	4	.013
$e/+ \times +$	172	2	.012
$+/+ \times +$	246	2	.008
$+/+ \times e$	393	0	.000
$+/e \times e$	950	5	.005
$+/e \times +$	471	3	.006

TABLE 4

Inhibition of mosaic production by the mutant long

Cross (<i>e/e</i> ♀ × <i>e</i> ♂)	♀ ♀	Mosaics	M/♀
<i>l/l</i> × <i>l</i>	1029	0	.000 ...
<i>+/+</i> × <i>l</i>	536	25	.050 ± .009
<i>+/l</i> × <i>+</i>	516	8	.015 ± .005
<i>l/l</i> × <i>+</i>	661	0	.000 ...
<i>l/+</i> × <i>+</i>	508	8	.016 ± .005
<i>l/+</i> × <i>+</i>	513	11	.021 ± .006
<i>+/+</i> × <i>+</i>	1079	43	.040 ± .006

did not carry the *ebony* mutant. Whether or not the sperm carried the *ebony* mutant was not important in the production of mosaics. *ebony* is, therefore, recessive to wild type in its ability to produce mosaics.

During these studies in which *ebony* was combined with other genes, it was found that a mutant gene, *long* (antennae), inhibited the production of mosaics from *e/e* females. A series of crosses was made in which all of the females were *e/e* and all of the males were *e. ebony*, *long* females crossed to either *ebony*, *long* or *ebony* males produced no mosaics (Table 4). *ebony* females that were heterozygous for *long* (*e/e* *+/l*) produced mosaics, but at a lower frequency than *ebony*, *not-long* (*e/e* *+/+*) females. The removal of the mutant *long* from *e/e l/l* females permitted the production of mosaics to increase to .040 ± .006 (Table 4). A summary of mosaic frequencies from crosses in Table 4 showed that no mosaics were produced to 1690 females from *ebony* mothers that were homozygous for *long*; 27 mosaics to 1537 females were produced from *ebony* mothers that were heterozygous *long* (.017 ± .003); 68 mosaics to 1615 females were produced from *ebony* mothers that did not carry the mutant *long* (.043 ± .005). It is clear from these data that the mutant *long* inhibits the production of mosaics from *ebony* (*e/e*) mothers.

In the experiments reported here, mosaics were observed among the progeny from crosses involving *ebony* females. P. W. WHITING (1932), however, obtained most of his mosaics from unmated heterozygous females. We, therefore, synthesized a stock in which the *ebony* females were heterozygous for four mutants (small wings, *sw*; white eyes, *wh*; lemon body color, *le*; orange eyes, *o*) (Table 5). Among the mated females, aberrant types were found. Among the

TABLE 5

Aberrant progeny from ebony mothers

Cross (♀ × ♂)	Gyn. ♂ ♂	Zyg.		Aber. types	Aber./♀
		♀ ♀	♂ ♂		
<i>e/e sw/sw wh/wh</i> × <i>e le o</i>	825	714	29	18	.025 ± .006
<i>e/e le/le o/o</i> × <i>e sw wh</i>	120	73	10	2	.027 ± .019
<i>e/e sw/+ wh/+ +/le +/o</i> × <i>e le o</i>	203	215	0	7	.033 ± .012
<i>e/e sw/+ wh/+ +/le +/o</i> (unmated)	3,005	0	0	0	.000 ...

TABLE 6

Nuclear origin of progeny from crosses of ebony females

Type	Nuclear origin	No. of progeny	Freq. among aberrant types	Freq. per ♀	Freq. per 10,000 ♀♀
gynogenetic ♂	G	4714			..
zygogenetic ♀	Z	4221			..
zygogenetic ♂	Z	163			..
androgenetic ♂	A	28	0.124	0.0066	66
mosaics	G + A	11	0.048	0.0026	26
	Z + A	88	0.389	0.0208	208
	Z + G	50	0.222	0.0119	119
	Z + G or A	11	0.048	0.0026	26
	Z + G + A	14	0.062	0.0033	33
	Z + Z	10	0.045	0.0024	24
	Z + Z + G	4	0.018	0.0010	10
	Z + Z + A	1	0.004	0.0002	2
	uncertain	9	0.040	0.0021	21
Total aberrant wasps/♀ ♀		226	1.000	0.0535	535

unmated females, there were no mosaics among 3005 male progeny! This indicates that aberrant types arose only from mated females; for every aberrant type there was a sperm involvement.

From various crosses involving mutant markers and in which the females were homozygous *ebony*, aberrant types were obtained (Table 6). These aberrant types were either androgenetic males or mosaics (including sex mosaicism). A total of 226 aberrant wasps were scored in these experiments (0.0535 per female). These wasps were classified into groups to show their nuclear (parental) origin (Table 5). Most of these types showed a diploid nucleus of zygogenetic origin and in addition a haploid nucleus of gynogenetic and/or androgenetic origin. In some cases, fertilization of two genetically different female pronuclei occurred. Some types were mosaic haploid males in which one part of the body was gynogenetic and the other part was androgenetic, indicating that a sperm had penetrated the egg but had not united with the egg pronucleus. Androgenetic males were also observed and indicated that the female pronucleus was not functional in development. The frequency of each aberrant type among total aberrant types, the frequency per female, and the frequency per 10,000 females are shown in Table 6.

DISCUSSION

The literature dealing with mosaicism in insects indicates that not only is the production of mosaics under genetic control but also that specific modifications in maturation and fertilization are related to specific mutants. In the present work on *Habrobracon* not just one type, but apparently a variety of aberrant types, were produced. But, since they all seem to be related to a single gene locus, we need to ask whether there is modification of a common developmental event

that causes all of these types to appear. The rather high frequency of the total aberrant types (5 per 100 females) enables one to determine the relative frequency of the different aberrant types.

Certain general rules seem to limit and guide the interpretation: (1) the mother must be homozygous for *ebony*; (2) no mosaics are produced from unfertilized eggs; (3) every aberrant type has inherited a paternal genome; all show a sperm involvement; (4) the principle of cell autonomy holds.

The largest group among the aberrant types (72 per cent) was haplo-diploid mosaics in which the diploid part was zygogenetic and the haploid part either gyno- or androgenetic, or both. It is not possible from the data presented to determine whether (1) nuclear union occurred before cleavage and that extra egg and sperm pronuclei were functional, or (2) union occurred among cleavage nuclei arising from a single egg and sperm pronucleus. Which of these interpretations holds is critical, but this can perhaps be settled experimentally by crosses involving appropriate gene markers. Since no mosaics were produced from unmated mothers, it is more difficult to consider that extra female pronuclei are involved. In addition, the Z + G and Z + A types occurred in approximately equal numbers. It is difficult to think of a common mechanism that would permit both extra female pronuclei and accessory sperm to be involved in mosaic production since the former would depend upon forces that permit more than one female pronucleus to migrate to the interior of the egg while the latter would call for a mechanism that controls the number of sperm that enter the egg.

About 5 per cent were diplo-diploid mosaics in which part of the organism was zygogenetic female and part was zygogenetic male. There are clearly two genetically different egg pronuclei, each of which unites with a sperm pronucleus. Whether we are dealing with dispermy or cleavage male nuclei is not clear.

About 2 per cent were diploid-diploid-haploid mosaics. There are at least two genetically different egg pronuclei each of which has united with a sperm pronucleus and in addition a haploid nucleus of either gynogenetic or androgenetic origin. This type appears to be a combination of haplo-diploid and diplo-diploid types. If, however, these are independent events, diploid-diploid-haploid types should be expected with a frequency of 1 per 10,000 females. The actual frequency, however, is 12 per 10,000 females.

About 5 per cent were haplo-haploid mosaics of gyno- and androgenetic origin. Both an egg and a sperm pronucleus were present but they did not unite to form a diploid nucleus.

About 12 per cent were androgenetic males. A sperm pronucleus was functional in development but there was no functional egg pronucleus.

It is possible that in the production of all of the aberrant types there is an effect on the positioning of the pronuclei in the egg. The *ebony* mutant by conditioning the oocyte, may hinder the ability of female pronuclei to migrate into the interior of the egg to unite with the sperm pronucleus. In the production of androgenetic males, the female pronuclei have not moved away from the egg

cortex and are therefore destroyed. In the production of gynogenetic-androgenetic haploid male mosaics, the female pronucleus does not migrate fast enough into the interior of the egg to unite with the sperm pronucleus, but since it is removed from the egg cortex it undergoes cleavage. The slower migration of the egg pronucleus may disrupt the timing of developmental events so that the pronucleus divides before entering a special region of the egg where union of nuclei of maternal and paternal origin may occur. Likewise, the sperm pronucleus may divide before the delayed egg pronucleus enters the region of the egg where union can occur. A small proportion of mosaics, however, clearly showed that two genetically different female pronuclei are functional in the egg. This is more difficult to explain but could also involve an effect of the *ebony* mutant on the migration of pronuclei. Further work will involve more refined genetic analysis and detailed cytological study of newly laid eggs.

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SUMMARY

Habrobracon females homozygous for the mutant *ebony* produce about 5 per cent mosaic and androgenetic progeny among fertilized eggs. These aberrant types are not produced by unmated *ebony* females. Every aberrant type shows a sperm involvement. The aberrant types are zygotenetic-androgenetic mosaics, zygotenetic-gynogenetic mosaics, zygotenetic-gynogenetic-androgenetic mosaics, zygotenetic-zygotenetic mosaics, zygotenetic-zygotenetic-androgenetic or- gynogenetic mosaics, gynogenetic-androgenetic mosaics, androgenetic males. —It is suggested that the occurrence of these diverse types of unusual progeny can be explained by assuming that the *ebony* mutant acts to delay the migration of the female pronucleus. This delay may allow for precocious cleavage of the egg or sperm pronucleus or cleavage before the union of egg and sperm nuclei. —The present work and other reports indicate that there are specific genes for specific steps in oogenesis and fertilization. This indicates that the process of fertilization can be studied from the standpoint of developmental genetics.

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