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Haploid intersexes in *Mormoniella**

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1. INTRODUCTION

In the parasitic Hymenopteron *Mormoniella vitripennis* (Walker) males develop from unfertilized eggs and females from fertilized eggs. Consequently, in normal stocks males are haploid and females are diploid. The genetic mechanisms underlying this system of sex determination are as yet unknown, however. No sex locus containing multiple complementary alleles, such as that found in *Habrobracon juglandis* (Ashmead) by P. W. Whiting (1943*a*), has yet been discovered in *Mormoniella*. Further, close inbreeding does not result in sterile diploid males (sex homozygotes in the *Habrobracon* system); the diploid males that have been found in *Mormoniella* (P. W. Whiting, 1960*a*) arose through cytogenetic disturbances of an unknown nature, are highly fertile, and sire triploid daughters through the production of diploid sperm.

Haploid intersexes from unfertilized eggs have been found in *Habrobracon* on three occasions (Whiting, 1943*b*; Whiting and Starrells, 1950; von Borstel, 1960*a*). Genetic data indicate that all three occurrences were due to mutations at the same locus. Phenotypically these intersexes were male anteriorly, grading to female posteriorly: antennal length, microchaetal dispersion on the wings, and behaviour were typically male, whereas abdomens contained normal stings and sensory gonapophyses, internal poison sacs, and associated glands. Abdominal sclerites were of female type posteriorly, grading towards maleness anteriorly and gonads resembled poorly developed ovaries. The distribution of wing microchaetae suggested that thoraces were probably male. Unfortunately, analysis of gene action in causing the intersexuality was prevented by loss of the mutations; interpretations of the significance of the intersexes therefore differ among the authors cited above.

This paper reports the occurrence of haploid intersexes in *Mormoniella*. The intersexuality appears to result from gene mutation, and bears a close resemblance phenotypically to the condition found in *Habrobracon*.

2. DESCRIPTION AND ANALYSIS

The intersexes were found among the progeny of a single unmated female which had arisen from the cross of oyster-white eyed (*oy*–NH) females by wild-type males. The wild-type males had been exposed to 3000 r. as part of a study of X-ray induced visible mutations in mature sperm. The gene *oy*–NH is an R-locus recessive to wild

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type and causes colourless eyes. Its gene formula (Whiting, 1960*b*) is *oy.st*. The female which produced the intersexes was phenotypically wild type; her progeny segregated as 32 normal males with wild-type (red-brown) eyes: 46 normal males with oyster-white eyes: 29 intersexes with wild-type eyes: 31 intersexes with oyster eyes. The culture contained no females, as expected since the parent female was unmated.

The segregation above gives no convincing evidence of genetic linkage between the intersexuality and the R-locus; assuming that the parent female was heterozygous for both intersexuality and eye colour and that these traits were linked in the repulsion phase, the data show a genetic distance of about 45 map units between the traits. The intersexuality may be associated with slightly lowered viability, since the counts shown above give a total of 78 normal males: 60 intersexes. No further genetic analysis of the intersexes was possible since the parent female was dead when the culture was examined, the intersexes did not mate, and the normal brothers of the intersexes did not transmit the trait. All normal brothers of the intersexes were crossed, in groups of five, to wild-type females; daughters from the cross were normal and produced no intersexes when set virgin.

Phenotypically, the intersexes showed a mixture of male and female traits. They were typically male in the colour and length of the antennae, eye colour, and body colour. The wings were intermediate in length, but were approximately as broad as those of normal females. Female genitalia and an ovipositor were present, and the internal stinging apparatus appeared to be identical to that of a normal female. The gonads resembled poorly differentiated ovaries, and on examination in aceto-orcein stain showed no evidence of oögonial cells or later stages in oöcyte formation. The gradation from maleness anteriorly to femaleness posteriorly is similar to that found in the *Habrobracon* intersexes (von Borstel, 1960); one difference is in the wings, which are intermediate in appearance in *Mormoniella*.

The intersexes were divided into two groups, each group containing both oyster-eyed and wild-type individuals; one group was placed in a vial with normal virgin females and the other with normal, freshly-enclosed males. A third vial contained sisters of the normal virgin females and brothers of the normal males which were placed with the intersexes; mating occurred immediately in this third vial, but no mating activity could be observed over a period of 10 hours in either vial containing intersexes. After 24 hours, *Sarcophaga bullata* pupae were put in the vials with the intersexes; these pupae were not stung or parasitized in the vial containing no normal females, and periodic checks revealed no apparent interest in the hosts on the part of any of the intersexes. The behaviour patterns of the intersexes were therefore typical of neither normal males nor of normal females, and the intersexes did not stimulate mating activity on the part of normal males. This neutral pattern of behaviour is in strong contrast to the typically male behaviour of the *Habrobracon* intersexes.

3. DISCUSSION

Some characteristics of the *Mormoniella* intersexes resemble those observed in the *Habrobracon* intersexes, but others are different. In each type a gradient from anterior maleness to posterior femaleness was apparent and the reproductive system resembled that of a normal female except that ovaries were rudimentary. In *Habrobracon*, however, the wings and behaviour pattern are typically male, whereas in *Mormoniella* the wings are intermediate and the mating reaction is absent. These differences may indicate (P. W. Whiting, personal communication) that the *Mormoniella* intersexes are somewhat less masculinized than those of *Habrobracon*; the differences in behaviour pattern may reflect differing effects of the intersex genes on the brain, which influences mating patterns in *Habrobracon* (Whiting, 1932, 1943*c*).

Although it was not possible to examine the chromosomes of the intersexes, the rather fragmentary genetic evidence indicates that, as in *Habrobracon*, the intersexuality resulted from gene mutation and not from chromosome aberration. If the intersexes resulted from a translocation involving a sex locus of the *Habrobracon* type, the frequency of intersexes among the viable male progeny of a heterozygous female should be about 0·33 and some of the normal brothers of the intersexes should transmit both the intersexuality and the partial sterility (von Borstel, 1960). The frequency of intersexes, however, was 0·435 and the female progeny of the normal brothers did not produce cultures containing intersexes; the egg hatchability counts needed to test partial sterility were not made. The low probability that the intersexes result from aneuploidy is indicated by the normally high inviability of aneuploids and the absence of intersexes among the progeny of triploid females (Whiting, 1960). On the other hand, the data are consistent with the assumption that the intersexuality resulted from mutation of a gene at a locus either loosely linked with the R-locus or segregating independently of it.

In contrasting mutations giving intersexuality of *Drosophila* with those giving *Habrobracon* intersexes, von Borstel (1960) has concluded that in *Drosophila* each mutation has shifted genetic females in the direction of maleness, whereas in *Habrobracon* and *Mormoniella* genetic males have been shifted toward femaleness. This generalization, however, is in contrast to the argument (Whiting and Starrells, 1950) that since in the *Habrobracon* intersexes the structures which differentiate during early embryological stages are female, whereas those which differentiate later are male, the intersexes start development as females and are thus basically haploid females. The same reasoning could be applied to the *Mormoniella* intersexes reported in this paper, with the additional argument that the change in developmental pattern occurs during later stages in *Mormoniella*, or is incomplete in some tissues.

Although the action of intersex genes in *Drosophila* contrasts strongly and consistently with the action of those in *Mormoniella* and *Habrobracon*, the assumption that an individual containing the chromosomal complement characteristic of a male (or female) must therefore be genetically a male (or female) is not yet proven. On the other hand, failure to establish any of the Hymenopterous intersexes in stock has prevented the embryological studies needed to test the hypothesis of Whiting and Starrells. It is, in fact, likely that the bases of intersexuality in insects will remain obscure until more is known about the biochemistry of sex determination.

SUMMARY

Intersexual individuals occurring among the progeny of a single virgin female *Mormoniella* are described and compared with similar cases of intersexuality described for *Habrobracon*. Evidence is presented in support of the hypothesis that the intersexuality resulted from a mutation at a single locus.

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