MEIOTIC DRIVE AND VISIBLE POLARITY IN DROSOPHILA SPERMATOCYTES

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THE model for meiotic drive presented by PEACOCK and ERICKSON (1965) demands that an intracellular differentiation exists at the time of the first meiotic division in spermatocytes. As a result of this differentiation, one of the spindle poles at anaphase I will lead to the formation of two functional sperm, while the other pole will yield two nonfunctional sperm. This model can be used to explain cases of meiotic drive, such as Segregation-Distorter (*SD*) (SANDLER, HIRAZUMI, and SANDLER 1959), if one member of a chromosome pair moves to the functional pole in a nonrandom fashion, and is thereby preferentially included in the functional gametes.

While the model provides a satisfactory explanation for the meiotic drive of *SD* and of the *sc*⁴-*sc*⁸ X chromosome (PEACOCK 1965), as well as the "sex ratio" phenomenon in the *obscura* group of *Drosophila* (NOVITSKI, PEACOCK and ENGEL 1965), the polar differentiation required by the model was not demonstrated directly. In the case of *SD*, the hypothesis of functional and nonfunctional poles at anaphase I is based on the observations that the *SD* chromosome always separates reductionally from *SD*⁺ at the first meiotic division, that spermiogenesis is completely normal, and that only half of the sperm, the half that carry the *SD* chromosome, are able to fertilize eggs. The same conclusion was demanded in the case of *sc*⁴-*sc*⁸ and the "sex ratio" condition where inequalities in the frequency of recovery of reciprocal classes of gametes were coupled with cytological observations demonstrating that reciprocal classes were formed with strict equality. In none of these instances was there a visible difference associated with the spindle poles of spermatocytes which would permit a direct morphological confirmation of their inequality.

First division spermatocytes in early pupae of some strains of *Drosophila* frequently contain a mass of cellular inclusions at one end of the cell (PEACOCK and ERICKSON 1964). The behaviour of these "granules", which appeared to be bacteria, indicated that they responded to an intracellular differentiation associated with meiosis. Polarization of the granules was apparently restricted to meiotic cells, yet randomly distributed granules were also present in connective, nutritive, and spermatogonial cells. These authors suggested that the incorporation of cytologically heteromorphous homologues known to exhibit meiotic drive into a "granule infected" cytoplasm would permit direct cytological verification

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of whether or not chromosomal and granule systems respond to a cytoplasmic gradient.

The present paper reports the results of such a study. It is concluded that the behavior of the granules (which are shown to be bacteria) is correlated with the behavior of a meiotically driven chromosome pair, and that both systems are probably responding to the same intracellular polarity. Furthermore, the simplest hypothesis requires the existence of a nonfunctional class of gametes that is determined at the first meiotic division.

MATERIALS AND METHODS

The sex chromosome pair, *Ins (1) sc⁴-sc⁸, γ and γ^+ Y*, consists of visibly different homologues which exhibit a strong meiotic drive (PEACOCK 1965). To obtain these chromosomes in cytoplasm containing granules, *sc⁴-sc⁸, γ/γ^+ Y* males were mated with females from stocks known to carry granules. Three such stocks were used: γ (b-32, Oregon), *cn bw* (Madison) and γ ; *cn bw* (derived from the γ and the *cn bw* strains). Crosses between *sc⁴-sc⁸, γ/γ^+ Y* males and infected females yield two types of F_2 males: those containing the *sc⁴-sc⁸* chromosome, and those containing a normal X chromosome. The *sc⁴-sc⁸* chromosome can be recognized by its visible difference in size from the normal X chromosome (the *sc⁴-sc⁸* chromosome lacks most of the proximal heterochromatin), and by the much lower frequency of XY bivalents at metaphase I. Males containing the normal X served as controls. All cultures were kept at 25–26°C, since the *sc⁴-sc⁸* chromosome exhibits a higher rate of non-disjunction at this temperature than at 18°C (ZIMMERING 1963).

Testes from late third-instar larvae, prepupae, and very early pupae were dissected in 0.7% saline and transferred to aceto-orcein. Squashes were examined with phase optics, and anaphase I divisions in cells containing granules were scored.

Sibs of the F_2 males used for cytology were mated to γ ; *cn bw* females for a genetic check of the drive characteristic of the *sc⁴-sc⁸* chromosome. Further data were obtained from a similar cross made at a later date.

The fine structure of the granules was determined by electron microscopy of pupal testes of γ ; *cn bw* males. The testes were dissected in 2.5% glutaraldehyde in 0.05 M sodium phosphate buffer pH 7.2, and held at 4°C for 8 hr. After washing in buffer they were post-fixed in 1% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 7.2, dehydrated in alcohol, and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 200 electron microscope.

RESULTS

The nature of the granules: Thick sections of epon embedded testes were examined with phase optics. In some spermatocytes, granules, similar to those seen in the squash preparations, were found. Thin sections of these areas of embedded tissue were examined in the electron microscope. Electron micrographs (Figures 1 and 2) reveal the granules to be membrane bounded and to contain randomly dispersed ribosome-like granules. Also, fine strands similar to those found in structures containing deoxyribonucleic acid were seen in irregular open spaces in the ground substance (Figure 2). The size, shape, and structure of these membrane bounded bodies are typical of Rickettsiae or Bacteria (ANDERSON, HOPPS, BARILE and BERNHEIM 1965) and they will be broadly referred to as bacteria in this paper. Their profiles were not similar to those of normal cell organelles, such as mitochondria. The granules are not present in spermatocytes of granule-free flies and were only seen in the electron microscope in those

FIGURE 1.—A group of "bacteria" (B) in the cytoplasm of a primary spermatocyte ($\times 20,000$).

FIGURE 2.—A single "bacterium" in cytoplasm ($\times 90,000$). (R) ribosome-like granules, (CR) cytoplasmic ribosomes, (D) DNA-like fibrils, (M) membrane.

spermatocytes, of infected flies, which showed granules in thick sections observed in phase optics.

ERICKSON and ACTON (unpublished) have reported similar results from electron microscope studies. Insects have long been known to have symbiotic intracellular bacteria (BROOKS 1963), some of which are transmitted only through the female in the egg cytoplasm. Maternal inheritance of the granules originally suggested by PEACOCK and ERICKSON (1964), has been confirmed by more extensive data of ERICKSON and ACTON. WOLSTENHOLME (1965) has reported on similar bodies from the salivary glands, and other organs, of some strains of *D. melanogaster*.

Cytological results: The number of bacteria in the primary spermatocytes varied from male to male, and from cell to cell within the same testis. The actual numbers varied from 10–20 per cell to several hundred per cell; an accurate assessment of the upper limit was not possible. The only cells scored were those with a large enough population of bacteria to allow precise determination of the position of the bacteria with respect of the anaphase I poles (Figure 3). In general, this meant that a cell contained not less than 100 bacteria, and usually the numbers involved were several hundreds.

In about 80% of the cells scored, the bacteria were grouped at one end of the cell or the other. In the remaining cells, the bacteria usually were located laterally to and parallel with the meiotic spindle (Figure 4); or, in rare instances, the bacteria appeared to be equally distributed between the two poles. The cells with either lateral or equal distribution were scored as "lateral".

In most "polarized" cells the bacteria were distributed in an all and none fashion (Figure 5). Infrequently a few bacteria would occur at the pole opposite to the large aggregate of bacteria (Figure 6) but these cells could always be classified unambiguously. The lack of complete polarity in some cells might be due to the squash method of preparation but any such explanation is purely speculative.

Cells with pronounced polarity were scored as to the location of the bacteria with respect to the X, Y, XY or nullo poles. The results for the sc^4-sc^8 males and their normal sibs are given in Table 1.

In the disjunctional divisions of the sc^4-sc^8 males there is a preponderance of cells with the bacteria grouped at the Y pole, ($\chi^2 = 4.50$, $0.05 > P > 0.01$). In nondisjunctional divisions, there is an excess of cells containing bacteria grouped

TABLE 1

The location of intracellular bacteria in relation to chromosome segregation at anaphase I in Drosophila spermatocytes

Genotype of male	Disjunctional divisions				Nondisjunctional divisions			
	Chromosomes at "bacteria" pole X	Y	Lateral bacteria	Y/(X+Y)*	Chromosomes at "bacteria" pole 0	XY	Lateral bacteria	XY/(0+XY)*
$sc^4-sc^8, \gamma/\gamma + Y$	57	82	36	.590	24	59	29	.711
$+/\gamma + Y$	52	50	26	.490	0	0	0	.000

* These values are designated as "s" in the calculations of "p" (see text).

FIGURE 3.—Early anaphase I, nondisjunctional with both sex chromosomes moving towards bacteria-containing pole. FIGURE 4.—Late anaphase I, disjunctional with bacteria lateral to spindle. FIGURE 5.—Late anaphase I, nondisjunctional with both sex chromosomes at bacteria-containing pole. FIGURE 6.—Late anaphase I, disjunctional, bacteria at both poles, the majority at the Y pole.

at XY pole ($\chi^2 = 14.75$, $P < 0.001$). In the control sibs, the X and Y classes do not differ significantly. The proportion of cells with equal or lateral grouping of the bacteria is similar in both division types of *sc¹-sc⁸* males and in anaphases of normal males.

Genetic results: The results of the genetic experiments are given in Table 2.

TABLE 2

The recovery of gametes from crosses of sc^4-sc^8 and normal males to $y; cn\ bw$ females

Genotype of male		Disjunctional gametes			Nondisjunctional gametes		
		X ($y\ \bar{y}\ \bar{y}$)	Y ($+\ \bar{y}\ \bar{y}$)	X/(X+Y)*	0 ($y\ \bar{y}\ \bar{y}$)	XY ($+\ \bar{y}\ \bar{y}$)	0/(0+XY)*
$sc^4-sc^8, y/y+Y$	I	152	77	.664	129	9	.935
	II	731	338	.684	441	17	.963
$+/y+Y$	I	474	479	.497	2	0	1.000
	II	1899	1818	.511	0	0	0

* These values, which are measures of meiotic drive, are designated as "*t*" in the calculation of "*p*" (see text).

The relative proportions of gametes recovered in experiment I (in which the males used were sibs of dissected males) and experiment II (which used males of the same genotype, but was done several months later) are the same. These results show that disjunctional gametes containing an X chromosome are recovered preferentially with regard to their reciprocal classes. If there were no preferential recovery, the values would all approximate 0.5, as is the case for the disjunctional gametes of control males.

DISCUSSION

Relationships of the genetic and cytological data: A direct comparison of the genetic and cytological data from sib males can be made in the amount of non-disjunction. The frequency of genetic nondisjunction is 0.376, as compared to 0.390 measured cytologically. For control males the values are 0.002 and 0.000. The genetic and cytological results are not significantly different, and indicate that both methods are measuring the same meiotic event.

We now ask whether the cytologically observed distribution of bacteria is in any way related to the genetic recovery of a particular gamete type compared with its reciprocal type. (Only the results for cells showing polarization can be used for this analysis, since the "lateral" group provides no usable information.) In the disjunctional anaphase of sc^4-sc^8 males, the X chromosome is seen more frequently at the bacteria free pole, and the genetic data show that the X is recovered more frequently than the Y. Similarly, in the nondisjunctional cases, the nullo pole is usually bacteria free, and the nullo class of gamete has the higher genetic recovery. This implies that the bacteria tend to aggregate at the same meiotic pole as the chromosomes that are recovered in lower frequency. Therefore, the frequency of association of polarized bacteria with respect to one or the other reciprocal class should be a cytological measure of the genetic recovery of that class.

Meiotic drive: These results can be related to the hypothesis advanced to explain the meiotic drive of the sc^4-sc^8 system (PEACOCK 1965). This hypothesis was based on the suggestion of NOVITSKI and SANDLER (1957) that *Drosophila* regularly produced some nonfunctional sperm. PEACOCK proposed that the unequal recovery of complimentary gamete types, both disjunctional and nondis-

junctional, resulted from nonrandom movement of the X and Y chromosomes to the anaphase I poles. These poles were suggested to differ in some predetermined way such that one (the functional pole) could lead to the production of two sperm capable of fertilizing an egg, whereas the other (the nonfunctional pole) did not have this capability. It was established that unpaired X and Y chromosomes did exhibit nonrandom movement on the meiotic spindle, but there was no way of knowing if this was in relation to the supposed predetermined functional and nonfunctional poles because these could not be distinguished cytologically. The presence of bacteria in the spermatocytes permits this distinction to be made.

We have seen that the bacteria are not polarized at random with respect to the chromosome complement of the poles of the first division spindle. An estimate of the degree of nonrandomness of their location can be obtained from the equation:

$$s = pt + (1-p)(1-t)$$

where s is the observed frequency of occurrence of the bacteria with the Y chromosome in disjunctional anaphases, and with the X and Y chromosomes in non-disjunctional anaphases (Y/X+Y or XY/0+XY, see Table 1); and where t is the coefficient of meiotic drive for the X or 0 gametes from disjunctional and nondisjunctional gamete classes, respectively, (X/X+Y or 0/0+XY, see Table 2). On the hypothesis cited above t for disjunctional gametes represents the probability of the X chromosome proceeding to the functional pole at anaphase I. In the above equation p represents the probability that the bacteria will be located at the *nonfunctional* pole of the anaphase I spindle.

The variance of p can be shown by the use of large sample formulae (MODE and ROBINSON 1959) to be approximately:

$$[\text{var}(s) + \text{var}(t)(1-2p)^2]/(2t-1)^2$$

For disjunction in sc^4-sc^8 males, $p = 0.750 \pm 0.117$ and the value of p calculated for sc^4-sc^8 nondisjunction is 0.731 ± 0.055 .

The mean value of p is 0.741 ± 0.065 , indicating that the probability of the bacteria being located at the nonfunctional pole is the same in both disjunctional and nondisjunctional anaphases.

A meaningful value of p cannot be computed for the results from normal males because the cytological scores of association of the bacteria with the X or the Y chromosome are not significantly different from equality. This is the expectation regardless of the degree of correspondence between the location of the bacteria and the recovery of gametes, since the observed sex ratio approximates 1:1.

Polarity of the primary spermatocyte: We may conclude that the two poles of an anaphase I spindle are dissimilar (as indicated by the aggregation of bacteria) and that this dissimilarity is related to the probabilities of recovery of the various gametic types. The simplest interpretation of the genetic and cytological data reported in this paper is that the bacteria and the chromosomes are responding, independently, to the same intracellular gradient. This gradient affects the orientation of chromosomes in a bivalent, the direction in which univalents move at anaphase I, and the distribution of certain non-nuclear elements (in this case, bacteria).

Polar differentiation of this type was proposed in the original model for meiotic drive of *SD* presented by PEACOCK and ERICKSON (1965). They suggested that the definitive event was an orientation of the second chromosome bivalent at first metaphase, such that the *SD*-bearing chromosome proceeded to the functional pole at anaphase in the majority of primary spermatocytes. The same model also resolves the apparent contradiction between genetic and cytological results in the "sex ratio" phenomenon (NOVITSKI, PEACOCK and ENGEL 1965). The demonstration in the present work of a cellular polarity associated with meiotic drive serves as strong support for the model.

It should be stressed that the bacteria are not the causal agents in the *sc*⁴-*sc*⁸ meiotic drive system. The characteristic discrepancies in recovery between reciprocal classes of disjunctional and nondisjunctional gamete types reported previously (SANDLER and BRAVER 1954, ZIMMERING 1963, and PEACOCK 1965) are unaltered by the presence of bacteria in *sc*⁴-*sc*⁸ males. ERICKSON and ACTON (unpublished data) have also shown that the action of *SD* is the same in infected and noninfected males. Finally, there is no evidence to suggest that the bacteria themselves are responsible for the cytoplasmic polarity. The available evidence indicates that they are merely acting as cellular components which, because of their size and number, make visible an inequality of the meiotic poles that is normal and of general occurrence.

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SUMMARY

A meiotic drive system consisting of the visibly heteromorphic homologues, *sc*⁴-*sc*⁸, γ and γ^+ *Y*, was analyzed cytologically and genetically in males containing granular cytoplasmic inclusions in meiotic cells. Electron microscopy has revealed that the granules are Rickettsiae or Bacteria. The relative frequencies with which aggregates of these bodies associate with the alternate poles in disjunctional and nondisjunctional anaphase I cells bear a constant relationship to the frequencies of recovery of the reciprocal classes of gametes. It is concluded that the bacterial aggregates and the sex chromosomes are responding independently to a pre-existing cytoplasmic gradient in meiotic cells. This conclusion is in accord with the cytological model for meiotic drive proposed by PEACOCK and ERICKSON (1965), for it requires that the two most important features of the model, cellular polarity and regular nonfunctionality of one-half of the products of a single meiosis, are operative in the system studied.

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