An Autosomal Factor From *Drosophila arizonae* Restores Normal Spermatogenesis in *Drosophila mojavensis* Males Carrying the *D. arizonae Y* Chromosome

A. C. Pantazidis,*,† V. K. Galanopoulos*,† and E. Zouros†,‡,[1]

**Institute of Molecular Biology and Biotechnology (FO.R.T.H.), Heraklion 711 10, Crete, Greece, †Department of Biology, University of Crete, Heraklion 711 10, Crete, Greece, and ‡Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada*

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ABSTRACT

Males of *Drosophila mojavensis* whose *Y* chromosome is replaced by the *Y* chromosome of the sibling species *Drosophila arizonae* are sterile. It is shown that genetic material from the fourth chromosome of *D. arizonae* is necessary and sufficient, in single dose, to restore fertility in these males. In introgression and mapping experiments this material segregates as a single Mendelian factor (sperm motility factor, *SMF*). Light and electron microscopy studies of spermatogenesis in *D. mojavensis* males whose *Y* chromosome is replaced by introgression with the *Y* chromosome of *D. arizonae* (these males are symbolized as $moj Y^a$) revealed postmeiotic abnormalities all of which are restored when the *SMF* of *D. arizonae* is co-introgressed (these males are symbolized as $moj Y^a SMF^a$). The number of mature sperm per bundle in $moj Y^a SMF^a$ is slightly less than in pure *D. mojavensis* and is even smaller in males whose fertility is rescued by introgression of the entire fourth chromosome of *D. arizonae*. These observations establish an interspecific incompatibility between the *Y* chromosome and an autosomal factor (or more than one tightly linked factors) that can be useful for the study of the evolution of male hybrid sterility in Drosophila and the genetic control of spermatogenesis.

IN recent years there has been an effort from several laboratories to identify and map genetic factors in the genome of Drosophila that are responsible for interspecific hybrid sterility or inviability. WATANABE (1979) has identified a mutation in the 2R arm of *Drosophila simulans* that allows *D. simulans* males to produce both male and female progeny when crossed to *Drosophila melanogaster* females, a cross that normally yields only females. Similarly, HUTTER and ASHBURNER (1987) and HUTTER, ROOTE and ASHBURNER (1990) have described two mutations in the *X* chromosome of *D. melanogaster* (possibly of the same locus) that rescue normally inviable hybrids from crosses of *D. melanogaster* to *D. simulans*, *Drosophila mauritiana* or *Drosophila sechellia*. Several apparently single-locus factors causing male hybrid sterility have been detected and mapped in relation to known morphological markers in several species of Drosophila. One such locus was mapped to approximately 1 map unit from the *forked* locus on the *X* chromosome of *D. simulans* (COYNE and CHARLESWORTH 1986). Loci with similar effects were found to map close to two other *X*-linked markers of the same species [COYNE and CHARLESWORTH (1989); see also JOHNSON *et al.* (1992)]. These studies, together with those of WU and BECKENBACH (1983), NAVEIRA and FONTDEVILA (1986) and ZOUROS, LOFDAHL and MARTIN (1988) in different species-groups, suggest that a large number of loci on the *X* chromosome of Drosophila are potential sites for mutational change affecting male hybrid fertility.

The search for loci that affect hybrid fertility or viability is an extension of earlier studies which through chromosomal substitutions in backcross progeny sought to obtain estimates of the minimum number of loci involved in a given form of reproductive isolation, or to partition the effect of the isolation among chromosomes [see reviews by COYNE and ORR (1989a), ZOUROS (1989) and COYNE 1992)]. These studies have been important in our attempts to explain and assess the generality of some principles regarding the development of hybrid sterility or inviability, such as HALDANE's (1922) rule, and to generate new hypotheses and models for the origin and evolution of these forms of reproductive isolation (ZOUROS 1986; CHARLESWORTH, COYNE and BARTON 1987; COYNE and ORR 1989b). The identification of a chromosomal region or a gene that affects some form of reproductive isolation in F_1 hybrids or their progeny is only the first phase in the search for the genetic basis of reproductive isolation. The second phase must involve the identification of genes with which the identified gene or chromosome interacts in causing isolation. Since hybrids carry "normal" genes that in a homospecific background are compatible with viability, fertility or

[1] To whom all correspondence should be addressed.

normal mating behavior, the effects in hybrids can be understood only as disruptions of synergistic interactions between conspecific genes. Thus, at the genetic level the study of speciation amounts to the study of the nature, origin and evolution of intergenic incompatibilities that differentiate the probabilities of gene exchange among individuals. This appears to be true regardless of whether one subscribes to the isolation, recognition, or cohesion species concept (TEMPLETON 1989).

The literature of male sterility in Drosophila reports several incompatibilities between interspecific *X* chromosomes and autosomes [reviewed in ZOUROS (1989) and COYNE (1992)]. An incompatibility between the *Y* chromosome and an autosome was reported by VIGNEAULT and ZOUROS (1986) in the species pair *Drosophila mojavensis* and *Drosophila arizonae* [formerly *D. arizonensis*, RUIZ, HEED and WASSERMAN (1990)]. By backcrossing the fertile F_1 male from the cross "female *D. mojavensis* × male *D. arizonae*" to *D. mojavensis* they found that males homospecific for the fourth chromosome were sterile, whereas males heterospecific for that chromosome were fertile. The other autosomes were also checked and found to have no effect. From the segregation of sterility among males from backcrosses and a mapping experiment PANTAZIDIS and ZOUROS (1988) concluded that the effect of the fourth chromosome can be assigned to a chromosomal region that segregates as a single Mendelian factor (sperm motility factor, *SMF*).

Here we present results from an introgression experiment providing further evidence that this region segregates as a single Mendelian unit, even though its actual length (or any other property) remains unknown. Through repeated backcrosses we produced two types of males. The first type consisted of males that were *D. mojavensis* in every respect except that the *Y* chromosome was replaced by that of *D. arizonae* and was sterile. The second type had, in addition, the *SMF* of *D. arizonae* and was fertile. A comparative study of spermatogenesis of the two types showed that spermiogenic defects resulting from the introgression of the *D. arizonae Y* chromosome into *D. mojavensis* background are fully restored when the *SMF* of *D. arizonae* is co-introgressed.

MATERIALS AND METHODS

Drosophila stocks: We used the A875 stock of *D. arizonae* (donated to us by W. B. HEED) established originally as a multifemale line from a collection from Sonora, Mexico. The second stock used was *D. mojavensis* "brown-eye," a stock homozygous for a fourth chromosome eye-color recessive mutant (PANTAZIDIS and ZOUROS 1988). The two stocks were fixed for different electromorphs of the enzyme loci octanol dehydrogenase (ODH), malic dehydrogenase (MDH), phosphoglucomutase (PGM) and peptidase-2 (PEP-2), marking the second, third, fourth and fifth chromosome, respectively (VIGNEAULT and ZOUROS 1986). The only chromosome for which no marker was available was the sixth dotlike autosome, which comprises less than 1% of the genome (WASSERMAN 1962). We use the following symbolism: X, Y, II–VI to refer to *X*, *Y* and second to sixth chromosomes; *a*, *m* and *r* to refer to the specific origin of a chromosome (*arizonae*, *mojavensis* or *recombinant*, respectively); *br* and br^+ to refer to brown or red eye color phenotype, respectively. *D. arizonae* A875 and *D. mojavensis br* were used to obtain three types of males to which we refer as $mojY^a$, $mojY^aIV^{a/m}$ and $mojY^aSMF^a$. They were produced as follows.

***mojY*^{a} and *mojY*^{a}*IV*$^{a/m}$:** As shown in Figure 1, F_1 males from the cross "*mojavensis* females × *arizonae* males" were backcrossed to *D. mojavensis*. Half of the resulting males (B_1 males, for males resulting from backcross #1) were *br* and, invariably, sterile; half were br^+ and fertile. Male fertility was assayed as presence of motile sperm in living preparations of testes squashes (VIGNEAULT and ZOUROS 1986). br^+ males (which were also heterozygous for PGM) were singly backcrossed to *D. mojavensis*. After larvae appeared in the vials, the male parents were scored electrophoretically. Only cultures whose male parents were homozygous for ODH, MDH and PEP-2 were kept and pooled to produce the B_2 generation. Because there is no crossing over in males, this assured that in these parents the cytoplasm, the *X* chromosome, and the second, third and fifth chromosome pairs were all of *D. mojavensis* origin, and that the *Y* and one fourth chromosome were of *D. arizonae* origin. br^+ males from the selected vials were backcrossed to *D. mojavensis* to produce the B_3 generation. This type of crosssing is being continued in our laboratory, yielding always 50% *br* and sterile males and 50% br^+ and fertile. This indicates that the sixth chromosome is not required for male fertility (otherwise the segregation of fertile to sterile males ought to be 25% to 75%). It also makes it virtually certain that the sixth chromosome is fixed for the *D. mojavensis* homolog. The experiments described here were done between generations B_{38} and B_{40}. *br* males resulting from this scheme of crosses are designated as $mojY^a$ to indicate that they are *D. mojavensis* in all aspects except that their *Y* chromosome is of *D. arizonae* origin. The br^+ males are designated as $mojY^aIV^{a/m}$ to indicate that in addition to the *D. arizonae Y* chromosome these males are heterospecific for the fourth (*IV*) chromosome.

***mojY*^{a}*SMF*^{a}:** Figure 2 sketches a procedure that gradually leads to the production of $mojY^aSMF^a$ males. $mojY^aIV^{a/m}$ were first crossed to their br^+ sisters (products of the continuing crosses in Figure 1) which are heterospecific for the fourth chromosome. In these females there will be crossing over in the fourth chromosome because this chromosome is homosequential in *D. mojavensis* and *D. arizonae* (WASSERMAN 1962). From this cross we selected *br* males and established pair-matings with *D. mojavensis* (cross type 1). Some of these *br* males will be fertile having inherited the SMF^a from their mother (having the *br* phenotype assures that they inherited the SMF^m from their father and are, therefore, heterozygous for *SMF*). After larvae appeared in the vials, the male parents were scored for PGM and cultures with only a $PGM^{m/m}$ father were retained. The intent of this initial selection was to retain as parents $mojY^a$ males with a *D. arizonae* fourth chromosome that carried the SMF^a, but whose regions marked by the *br* and PGM loci were replaced through recombination with *D. mojavensis* material [these markers were shown to lie on both sides and at roughly equal recombination distances from *SMF*; PANTAZIDIS and ZOUROS (1988)]. Half of the daughters of males from cross type 1 are expected to carry the recombinant fourth chromosome, but they are indistinguishable from their homospecific (*D. mojavensis*) sisters. Thus, females emerging from

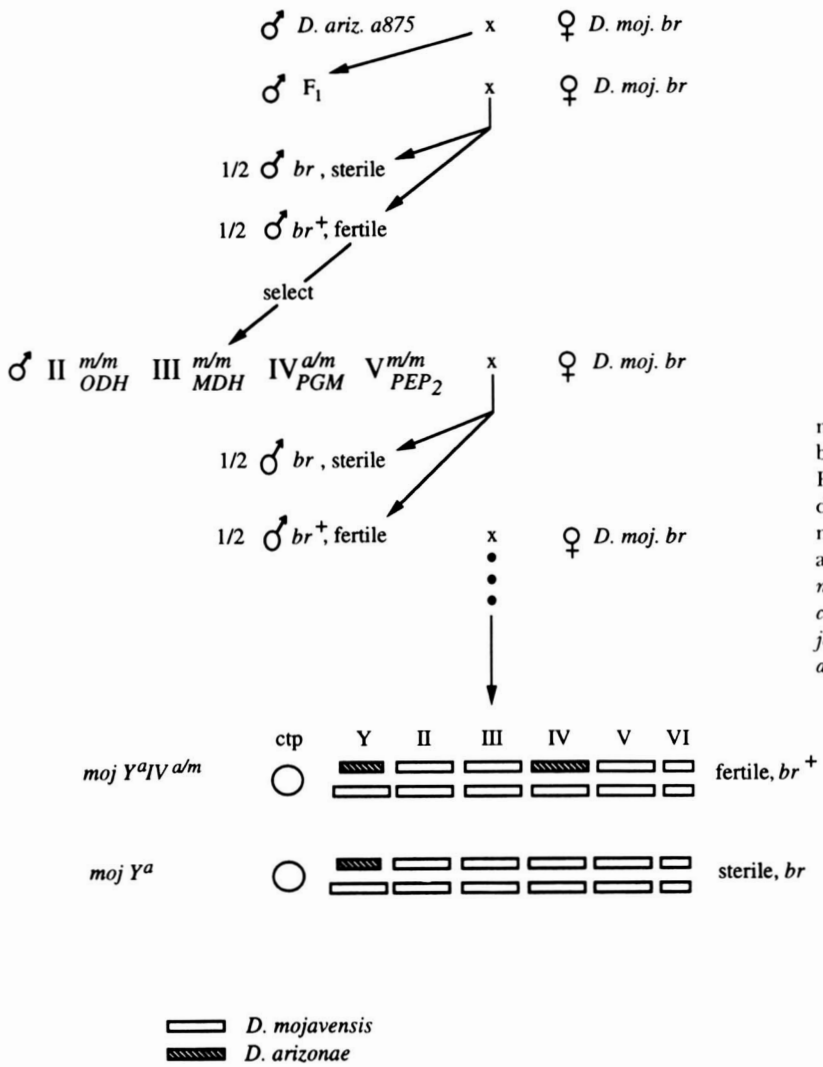

FIGURE 1.—Protocol of crosses for the production and maintenance of $mojY^a$ and $mojY^aIV^{a/m}$. *br* and br^+ stand for brown-eye and wild-type genotypes; ODH, MDH, PGM, PEP_2 for the enzyme loci octanol dehydrogenase, malic dehydrogenase, phosphoglucomutase and peptidase-2, marking chromosomes *II*, *III*, *IV* and *V*, respectively; *m* and *a* indicate chromosomes of *D. mojavensis* or *D. arizonae* origin, and *r* indicates recombinant chromosomes; *ctp*, cytoplasm; open designs, genetic material of *D. mojavensis*; hatched or filled designs, genetic material of *D. arizonae*.

the selected vials of cross type 1 were crossed en mass to *D. mojavensis* (cross type 2) and the emerging females were crossed to $mojY^aIV^{a/m}$ (cross type 3). Half of the males from cross type 3 were br^+ (and fertile) and were discarded. Among their *br* brothers some were fertile having inherited the SMF^a from their mother. In the following cross, which is equivalent to cross type 1, these chromosomes were retained. This crossing scheme continued for several rounds, each round consisting of crosses type 1 to type 3. The experiments described here were done at the 12th round.

Preparation of testes squashes: In these species males do not reproductively mature before the 5th day after eclosion. Testes from 3–5-day-old males were dissected in testis isolation buffer [TIB: 0.183 M KCl, 47 mM NaCl, 10 mM Tris-HCl, pH 6.8; HENNIG (1967)] under a Wild M8 stereoscope. After puncturing with a needle, testes were gently squashed in a drop of TIB on a microscope slide and the coverslip was sealed with nail polish. Phase contrast images of living cysts were recorded on Kodak Panatomic-X 35-mm film using a Leitz Dialux 20 EM light microscope equipped with incident-light fluorescence.

Preparation of 4′,6-diamidino-phenylindole dichloride (DAPI)-stained slides: Spermatid nuclei were detected in fixed preparations stained with DAPI, a highly sensitive fluorescent DNA dye (RUSSELL, NEWMAN and WILLIAMSON 1975; WILLIAMSON and FENNEL 1975). The preparation of DAPI-stained slides was done according to method I described by KREMER, HENNIG and DIJKHOF (1986). These slides were examined under the light microscope mentioned above. An ultra high pressure 50W lamp was used for illumination with the Leitz filter block A combination (BP 340-380, PL 430). Photographs were taken on a Kodak T-MAX 35-mm film.

Electron microscopy: Six- to eight-day-old males anesthetized on ice were dissected in Drosophila Ringer's solution. Testes were fixed in 2% glutaraldehyde/2% formaldehyde for 90 min at room temperature, washed in buffer containing 4% sucrose, postfixed in 2% OsO_4 for 60 min at 4°, dehydrated in ethanol and embedded in a modified Mollenhauer's resin (25 g of Epon-812, 20 g of Araldite-506, 60 g of dodecenylsuccinic anhydrite, 3 g of DMP-30). The fixation and washing buffer was 0.08 M sodium cacodylate at pH 7.4. Ultrathin sections were cut with glass knives using an LKB Ultratome V, collected on 200 or 300 mesh copper grids and successively stained with 7% uranyl acetate and 0.2% lead citrate aqueous solutions. Images from transverse sections of mature sperm bundles were obtained on Kodak electron microscope film 4489 (6.5 × 9-cm plates) using a JOEL 100 C Transmission Electron Microscope operated at 80 kV.

RESULTS

Segregation of sterility in backcrosses: The protocol of crosses shown in Figure 2 was designed to

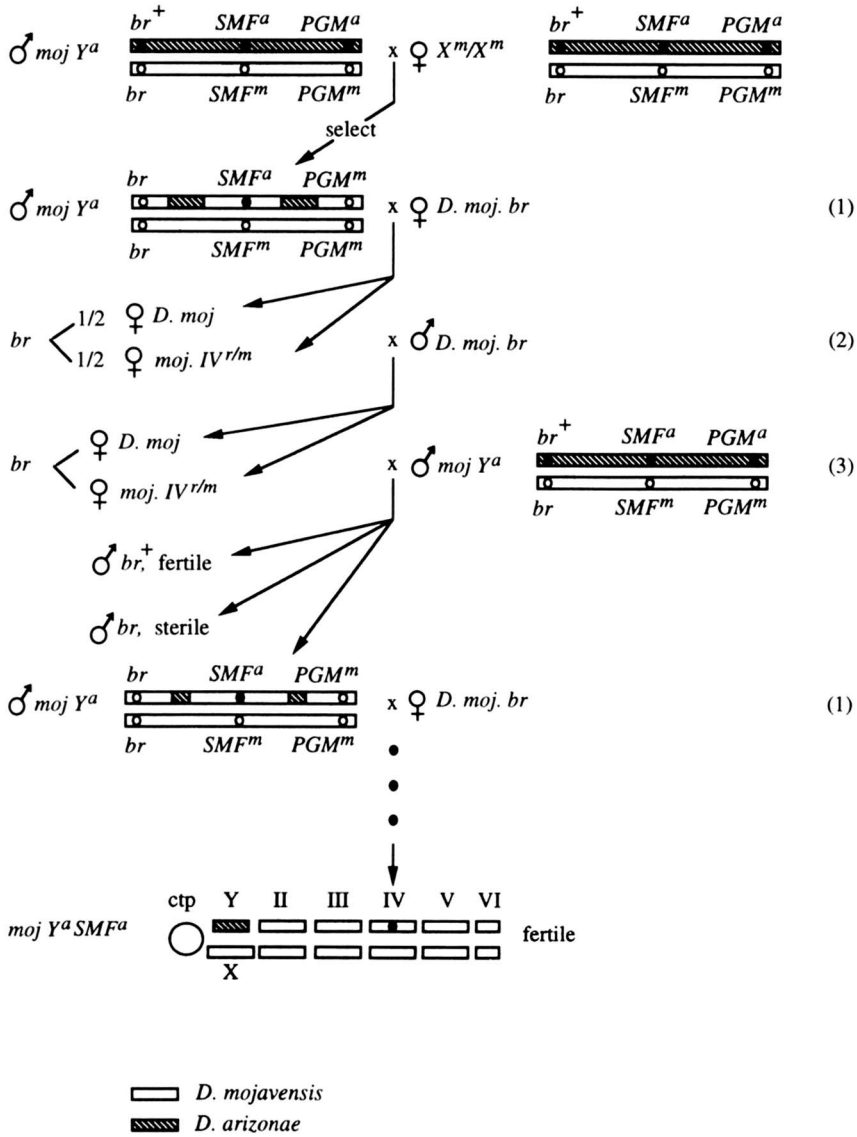

FIGURE 2.—Protocol for the production and maintenance of *moj*Y^a*SMF*^{a}. Notation as in Figure 1. See text for further explanation.

eliminate from the *D. arizonae* fourth chromosome material that is not necessary for fertility in males whose only other *D. arizonae* material is the *Y* chromosome. If there were several genes dispersed along the chromosome and all were necessary for fertility, then the whole chromosomal piece containing these genes would be selectively retained, so that carrying on the introgression scheme through repeated rounds would serve no purpose. In the lack of appropriate markers, evidence that the genetic material needed for fertility is all contained in a region that segregates as a single Mendelian unit can be obtained from the segregation of fertility among sons of females heterozygous for *SMF*. If fertility requires the simultaneous presence of several distantly located *D. arizonae* genes, crossing over in such females would decouple these genes so that only nonrecombinant chromosomes (and the recombinant ones in which the genes are retained in a *cis* configuration) would produce fertile sons. Their frequency would be less than 50%. Alternatively, if the fourth chromosome contained several duplicate genes each of which can restore fertility, more than 50% of progeny would be expected to be fertile. An one-to-one ratio would be predicted from the hypothesis of a single Mendelian factor. Evidence for this hypothesis was obtained by PANTAZIDIS and ZOUROS (1988) who examined the progeny from the initial cross of the protocol of Figure 2. From this cross one can identify all male progeny that have inherited the *SMF*^{m} chromosome from their father and a recombinant chromosome from their mother. In two different mass crosses the ratio of fertile to sterile sons was 1:1.

An independent segregation test was done in the first round of cross type 3 of Figure 2. For a simultaneous check that the results are independent of the strain of *D. arizonae* used, we started the protocol with a new *D. arizonae* stock (A874). We set 25 pair matings of the type 3 cross. An inspection of the protocol shows that the female parents in these matings will be of two morphologically indistinguishable types: those that will have inherited a complete *D. mojavensis* chro-

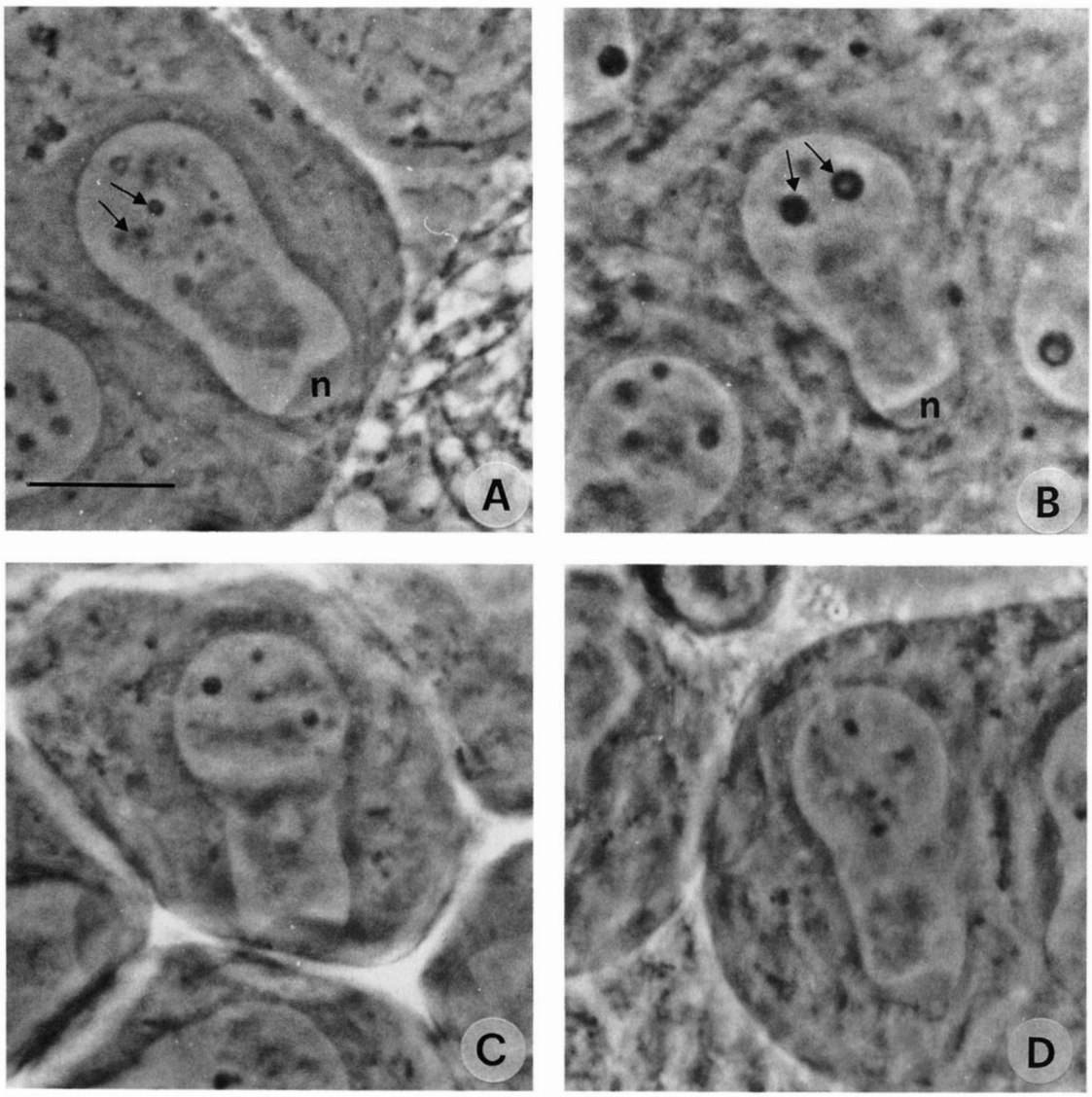

FIGURE 3.—Phase contrast images of nuclei of mature primary spermatocytes. (A) *D. mojavensis*, (B) *D. arizonae*, (C) $mojY^{a}$, (D) $mojY^{a}SMF^{a}$. The appearance of *Y*-chromosome loops differs between *D. mojavensis* and *D. arizonae* mainly in the number and size of the hollow granules (arrows) lying opposite to the nucleolus (n). No clear difference can be seen between the sterile $mojY^{a}$ (C) and the fertile $mojY^{a}SMF^{a}$ (D). Scale bar = 10 μm. Scale in B, C and D same as in A.

mosome from their mother and those that will have inherited a recombinant one. Among the latter some will not have inherited the SMF^{a} and some will. The latter will be the only ones that will produce fertile *br* sons (br^{+} sons will be fertile in all matings, having inherited the SMF^{a} from their father). Under the assumption of a Mendelian segregation of the *SMF*, the frequency of females that will produce fertile *br* is 0.25, and the ratio of fertile to sterile among these *br* sons is 1:1. Pure matings of *D. mojavensis* have low yields. A total of 627 progeny were obtained from the 25 matings. In 19 matings all *br* sons (total 120) were sterile. In 6 matings, some *br* sons were sterile and some fertile. Of the total of 43, 21 were sterile and 22 fertile (this ratio was also 1:1 within each mating; 3:4, 4:5, 5:3, 3:3, 2:4, 4:3). These results are fully consistent with the Mendelian hypothesis, both in regard to the ratio of females expected to produce fertile *br* sons and the fertile to sterile ratio among their sons. These observations cannot exclude, however, the possibility that *SMF* consists of a cluster of tightly linked genes.

Comparison of spermatogenesis: We have used a combination of light and electron microscopy to identify the major structural differences among males with different genotypes. The specific characteristics of spermatogenesis in *D. mojavensis* are presented elsewhere (PANTAZIDIS, ZOUROS and GALANOPOULOS 1992). The only difference between *D. mojavensis* and its sibling species *D. arizonae* is in the morphology of *Y* chromosome loops (see below). Of special interest is the comparison of three types of males: *D. mojavensis*, $mojY^{a}$ and $mojY^{a}SMF^{a}$. The first two differ in that the *Y* chromosome is of *D. mojavensis* origin in the first

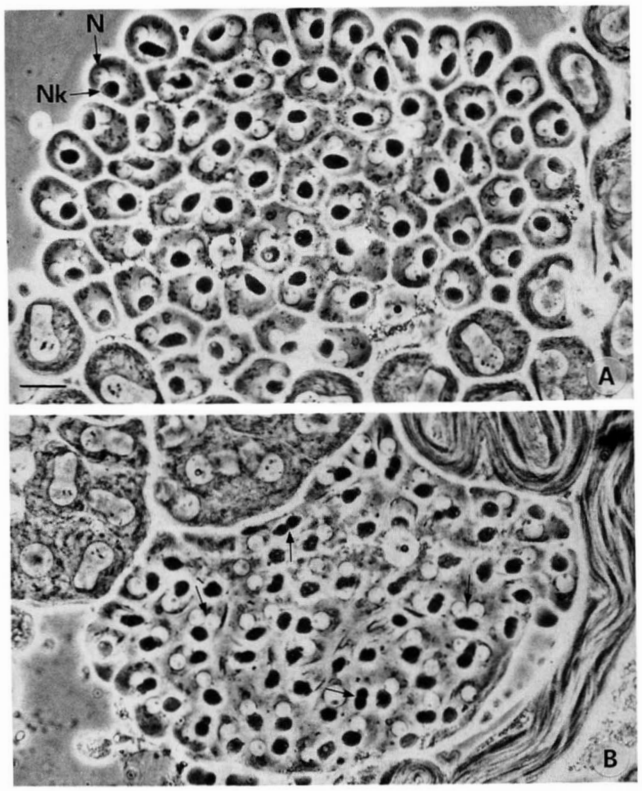

FIGURE 4.—Phase-contrast images of syncytia from the early post meiotic stage. (A) *mojY*^{a}*SMF*^{a}, (B) *mojY*^{a}. In *mojY*^{a}*SMF*^{a} each newly formed spermatid has one nucleus (N) and one mitochondrial mass (Nebenkern, Nk). Arrows in B point at meiotic products with two nuclei or two Nebenkerne. Scale bar = 20 μm. Scale in B same as in A.

and of *D. arizonae* in the second. The third differs from the second in that in one of the fourth chromosomes the region containing the *SMF* has been replaced by the corresponding piece of *D. arizonae*. The first and the third are fertile, the second is sterile. We have observed no major spermatogenic differences between the first and the third type. As a result our comparative study was confined mainly to the second and third type. In all cases our conclusions were based on multiple examinations of each developmental stage in different individuals of each genotype.

Spermatogenesis in *mojY*^{a} and *mojY*^{a}*SMF*^{a} begins normally with four rounds of incomplete mitotic divisions of a gonial cell which give rise to a cyst (syncytium) of 16 primary spermatocytes. One previously known difference between *D. mojavensis* and *D. arizonae* is the number and size of nuclear hollow granules appearing opposite to the nucleolus of the mature spermatocyte (HESS and MEYER 1968). According to these authors, these granules are part of the lampbrush loops of the *Y* chromosome, studied subsequently in detail in *Drosophila hydei* [reviewed in HENNIG (1985)] and *D. melanogaster* (BONACCORSI *et al.* 1988). We have confirmed these differences (Figure 3, A and B), but observations in our and in HENNIG's laboratory by one of us (A.C.P.) failed to produce a distinct and repeatable difference between *mojY*^{a} and *mojY*^{a}*SMF*^{a} (Figure 3, C and D). Also, we have not seen any other morphological difference in the premeiotic stages between these two genotypes.

The earliest disturbances of spermatogenesis in *mojY*^{a} can be seen immediately after the completion of meiosis. The products of meiosis can be easily distinguished in *mojY*^{a}*SMF*^{a} (Figure 4A), but are not well separated in *mojY*^{a} (Figure 4B). The number of nuclei and mitochondrial derivatives (also known as Nebenkerne) per cyst was recorded in squashes from twenty five individuals of each of the three genotypes: *D. mojavensis*, *mojY*^{a}*SMF*^{a} and *mojY*^{a}. In some individuals more than one cyst could be examined. The number of nuclei and mitochondrial derivatives was 64 in all 35 cysts from *D. mojavensis* and in all 33 cysts from *mojY*^{a}*SMF*^{a}. In 36 cysts of *mojY*^{a} examined, the number of mitochondrial derivatives was also 64, but the number of nuclei varied from 50 to 64, with an average of 55.50 ± 3.337. However, in many instances the 64 mitochondrial derivatives were not evenly distributed among the newly formed spermatids (Figure 4B), something that was never observed in the fertile genotypes. Another defect, common in male sterile mutants, was observed in the elongation stages. In *mojY*^{a}*SMF*^{a} the alignment of spermatids into a bundle was normal so that all nuclei converged on one region, the head region of the syncytium (Figure 5, A and C). In *mojY*^{a} the spermatids failed to align in a proper way and, as a result, their nuclei could be seen dispersed through the whole length of the bundle (Figure 5, B and D).

Ultrastructural observations by electron microscopy showed that spermiogenesis proceeds normally in *mojY*^{a}*SMF*^{a}, resulting in the formation of mature and individualized sperm (Figure 6, A and B). In contrast, the mitochondrial derivatives of *mojY*^{a} contained large quantities of trapped cytoplasm and had several foci of paracrystalline body accumulation. Irregularities in the number and pattern of axoneme microtubules were also observed, chromatin condensation did not reach completion and spermatids failed to individualize (data not shown). Whereas no structural differences in the development of spermatids were seen between *D. mojavensis* males and *mojY*^{a}*SMF*^{a}, the number of mature sperm per bundle in the latter genotype was found to be consistently less than 64 (Figure 6). In the examples of Figure 6, A and B, the numbers of sperm are 61 and 62, respectively. This observation prompted an examination of the number of mature sperm per bundle in tail cross-sections (as shown in Figure 6B) in four types of fertile males: *D. mojavensis*, *D. arizonae*, *mojY*^{a}*SMF*^{a} and *mojY*^{a}*IV*$^{a/m}$. The results from the analysis of variance are given in Table 1. There was little interindividual variation in the number of sperm per bundle in *D. mojavensis* and *mojY*^{a}-

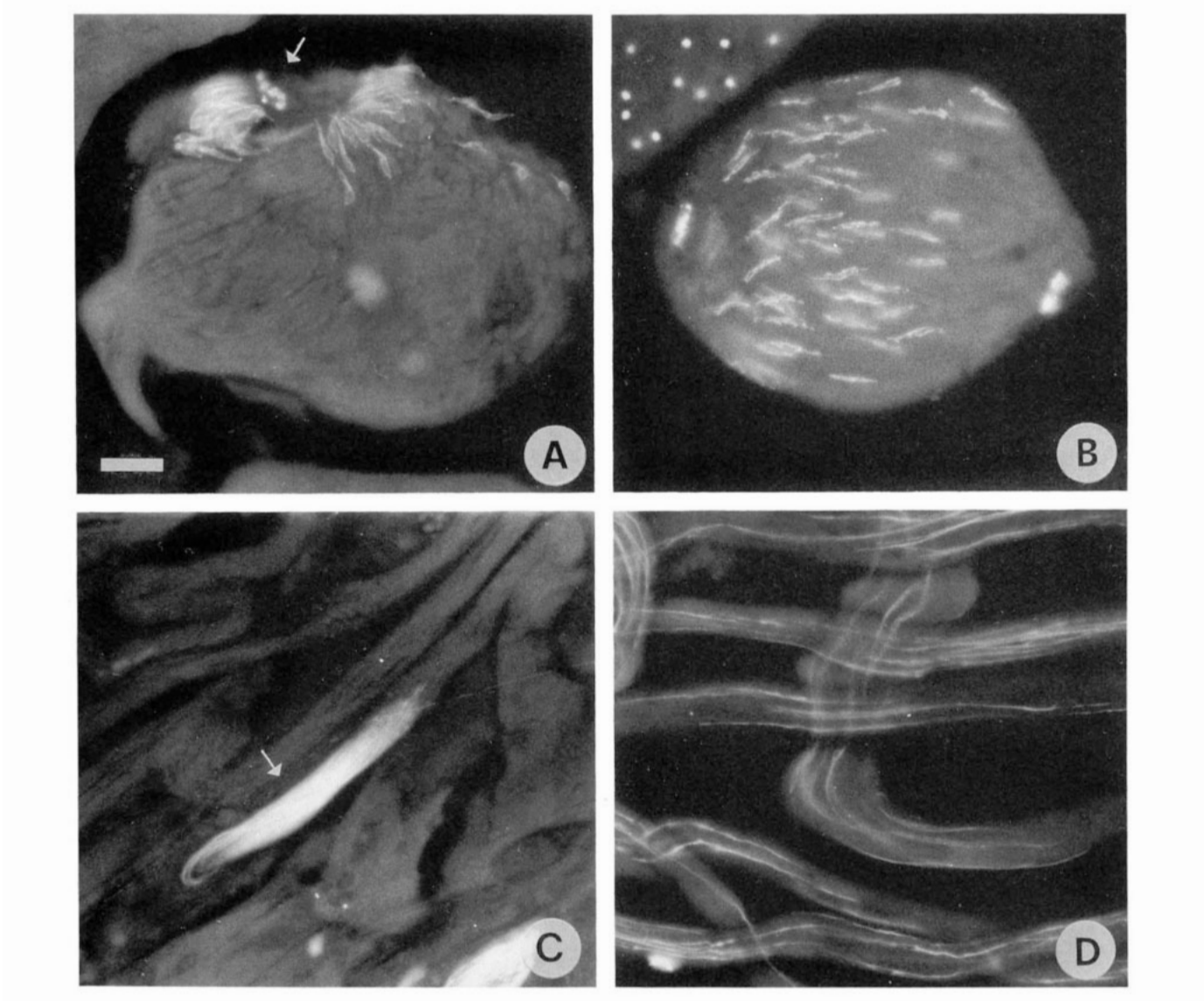

FIGURE 5.—Photographs of fixed and DAPI-stained testes showing the location of nuclei within the elongating spermatid bundles from *mojY*^{a}*SMF*^{a} (A and C) and *mojY*^{a} (B and D). Syncytia in A and B belong to the early elongation period, in C and D to the late elongation period. In *mojY*^{a}*SMF*^{a}, spermatids within the syncytium are aligned so that all nuclei converge on one region of the syncytium (arrow in A) and form a tight bundle (arrow in C). Spermatids in *mojY*^{a} fail to do so. Scale bar = 20 μm. Scale in B, C, D the same as in A.

SMF^{a} and the observed differences in *D. arizonae* barely approached statistical significance. In *mojY*^{a}*IV*$^{a/m}$; however, there were systematic differences among individuals (*e.g.*, in one individual the count was below 54 and in another above 60 in all three bundles examined). With the exception of *D. mojavensis/D. arizonae*, all among-genotype differences in the number of sperm per bundle were highly significant.

DISCUSSION

The main objective of this study was to contribute to the understanding of the evolution of male hybrid sterility. In Drosophila, this form of postzygotic reproductive isolation is much more common than female hybrid sterility (BOCK 1984; COYNE and ORR 1989a). In addition to following HALDANE's (1922) rule more faithfully than any other form of reproductive isolation (*e.g.*, hybrid inviability), it is also more often asymmetrical (affecting only the offspring from one of the two reciprocal crosses between two species) than female hybrid sterility, male hybrid inviability or female hybrid inviability (BOCK 1984). Asymmetry is a requirement for the evolution of incompatibilities between two or more heterospecific loci in many models of speciation (WU and BECKENBACH 1983; ZOUROS 1986). A compilation of allozyme distances between pairs of species showing hybrid sterility or hybrid inviability suggested that these two forms of reproductive isolation evolve at the same rate (COYNE and ORR 1989a). However, WU (1992) has pointed out that COYNE and ORR's statistic of allozyme distance is not a sensitive index of rate differences between these two forms of isolation and that hybridi-

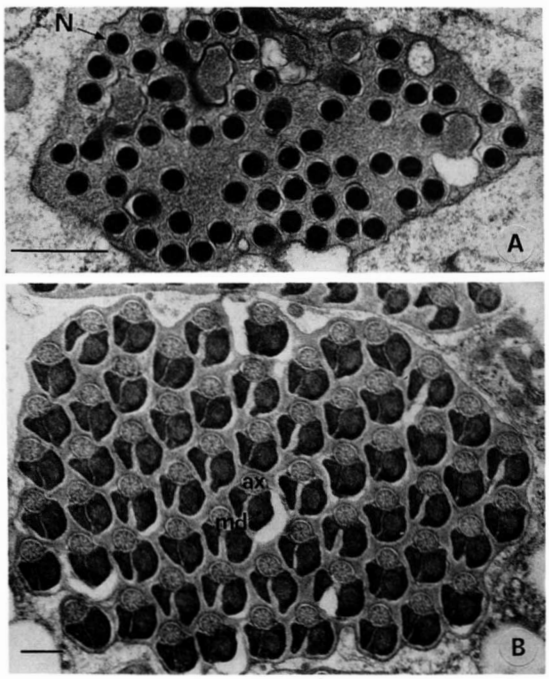

FIGURE 6.—Electron micrographs of cross-sectioned nuclei (A) and tails (B) from mature spermatozoa in two different bundles from *mojY^{a}SMF^a*. Sperm derived from the same syncytium remain together for a short period after the completion of the individualization process. The nuclei (N) are fully condensed and each tail is composed of one axoneme (ax) accompanied by two unequal in size mitochondrial derivatives (md). The number of nuclei in A is 61 and the number of tails in B is 62. Scale bars = 0.5 μm.

zation studies provide support for the view that hybrid sterility evolves faster than hybrid inviability. Direct evidence that male hybrid sterility has preceded inviability in the process of species divergence comes from the two known cases of hybrid rescue. Males emerging from the cross of *D. melanogaster* females to *D. simulans* males carrying the *Lhr* mutation (WATANABE 1979) or from the cross of *D. melanogaster* females carrying the *Hmr* mutation to males of *D. simulans*, *D. mauritiana* or *D. sechellia* (HUTTER and ASHBURNER 1987) are sterile. All these observations suggest that among the various forms of post-reproductive isolation, male hybrid sterility is the earliest to arise in most speciation events in Drosophila. It follows that studies of the genetic and developmental aspects of male hybrid sterility are important for the understanding of speciation, at least for those speciation events in which postzygotic isolation forces are more likely to have preceded prezygotic ones.

In *D. melanogaster* LINDSLEY and TOKUYASU (1980) estimate that, in addition to the six complementation groups of the *Y* chromosome, mutations at as many as 1250 autosomal and 300 *X*-linked loci may cause male sterility. The empirical evidence for the preponderance of *X*/autosome or *X*/*Y* incompatibilities leading to male hybrid sterility in Drosophila has been summarized in recent reviews (COYNE and ORR 1989b; ZOUROS 1989). There appears to be only one documented case of an autosome/autosome incompatibility. SCHAFER (1978) has shown that in males carrying the *X* chromosome of *D. hydei* and the *Y* of *D. neohydei* the combination *3H3N4H4H* (where *3* and *4* stand for the third and fourth chromosome, and *H* and *N* for *D. hydei* and *D. neohydei* origin) is always sterile. Since this combination is the only one that also causes sterility in males in which both sex chromosomes are of *D. hydei* origin, it is very likely that the incompatibility is not dependent upon the sex chromosomes.

An incompatibility between the *Y* chromosome of *D. arizonae* and the fourth chromosome of *D. mojavensis* leading to complete male sterility was shown by VIGNEAULT and ZOUROS (1986). Subsequent work by PANTAZIDIS and ZOUROS (1988) suggested that the effect of the fourth chromosome can be assigned to a single Mendelian factor, and experiments reported here corroborate this evidence. We have introgressed this factor onto a *D. mojavensis* background and examined the rescue of normal spermatogenesis in *D. mojavensis* sterile males carrying the *Y* chromosome of *D. arizonae*.

Spermatogenesis has been studied extensively in *D. melanogaster* [reviewed in LINDSLEY and TOKUYASU (1980)] and to a lesser degree in *D. hydei* (HENNIG 1985) and other Drosophila species (JAMIESON 1987). These studies have established several conclusions

TABLE 1

Number of mature sperm per bundle in four types of males

	D. mojavensis	*D. arizonae*	*mojY^{a}SMF^a*	*mojY^{a}IV$^{a/m}$*
No. of individuals examined	10	10	10	10
No. of bundles	42	41	46	32
Mean no. of sperm per bundle	63.59	63.48	62.13	57.03
Range	60–64	60–64	60–64	45–62
95% confidence intervals:	63.01–64	62.90–64	61.57–62.68	56.37–57.70
Probability that among-individual (within genotype) differences are not significant	0.362	0.046	0.226	0.0002
Among-genotype differences	All combinations are significant at the 10^{-7} level except the *D. mojavensis*/*D. arizonae* pair for which the probability is 0.615			

regarding the involvement of the *Y* chromosome in spermatogenesis. There are six complementation groups in the *Y* chromosome of *D. melanogaster* required for fertility (KENNISON 1981; GATTI and PIMPINELLI 1983) and as many as 16 in *D. hydei* (HENNIG 1985), yet the sensitivity of the *Y* chromosome to induction of sterility by X-irradiation or EMS treatment is as high as that of the *X* chromosome, which may harbor as many as 350 male fertility genes [references in LINDSLEY and TOKUYASU (1980)].

The earliest involvement of the *Y* chromosome in spermatogenesis is known to be in the development of the primary spermatocyte, where MEYER, HESS and BEERMANN (1961) first observed the presence of characteristic filamentous structures, now known to be giant lampbrush loops of the *Y* chromosome (HENNIG 1985; BONACCORSI *et al.* 1988). Our study has many points in common with previous studies by SCHAFER (1978) and HENNIG (1978), who backcrossed males from the cross "female *D. hydei* × male *D. neohydei*" to *D. hydei*. Males from this cross carrying no *D. neohydei* chromosomes (other than the *Y*) were completely sterile. We show here that an autosomal factor conspecific to the foreign *Y* chromosome can restore this chromosome's function, resulting in normal spermatogenesis. HULSEBOS, HACKSTEIN and HENNIG (1984) have observed that products derived from the *X* chromosome or the autosomes contribute to the morphology of the *Y* loops. The lack of an elaborate morphology of the *Y* lampbrush chromosomes in our species-pair seems to preclude gaining an insight on possible premeiotic lesions by focusing at the primary spermatocyte stage. Earlier attempts in the much more favorable species-pair *D. hydei* and *D. neohydei* (SCHAFER 1978; HENNIG 1978) have not produced a clear link between *Y*-loop morphology and spermiogenic abnormalities in males with an introgressed heterospecific *Y* chromosome. One could hypothesize that the *Y* chromosome contributes the loop backbone, but the actual loop morphology is mostly determined by products from the other chromosomes. This will explain why the $mojY^a$ and $mojY^aSMF^a$ loops resemble more those of the *D. mojavensis* than the *D. arizonae* loops (Figure 3). It is also compatible with the observation (SCHAFER 1978; HENNIG 1978) that loop formation does not preclude the subsequent appearance of massive spermiogenic abnormalities.

LIFSCHYTZ and HAREVEN (1977) and HARDY, TOKUYASU and LINDSLEY (1981) have reported that *Y* chromosome deletions cause abnormal movement and segregation of chromosomes during meiosis, so that the resulting cells do not have a complete haploid complement. It is possible that the meiotic products of $mojY^a$ males contain unbalanced sets of chromosomes, but it is unlikely these unbalances are responsible for the abnormalities appearing in the process of spermiogenesis. It is known that spermatids missing the entire set of chromosomes (nulliploid) can develop normally into motile spermatozoa (LINDSLEY and GRELL 1969).

The comparison of spermiogenesis of the fertile genotypes $mojY^aSMF^a$ and $mojY^aIV^{a/m}$ with that of *D. mojavensis* revealed no qualitative differences, but an important quantitative difference: the number of mature sperm per bundle was smaller in $mojY^aIV^{a/m}$ than in $mojY^aSMF^a$, which in turn was smaller than in *D. mojavensis* or *D. arizonae*. The difference between $mojY^aSMF^a$ and the pure-species genotypes is small (about one spermatozoon only), but consistent. Among 42 bundles of *D. mojavensis* examined, 10 had less than 64 spermatozoa. In *D. arizonae* these numbers were 41 and 9, respectively. Only 8 of the 46 bundles of $mojY^aSMF^a$ had 64 spermatozoa. Thus, the rescue of fertility in Y^a-carrying *D. mojavensis* by the introgression of the *D. arizonae SMF* is very nearly complete, but still not entirely so. The number of mature sperm per bundle is much smaller in males whose fertility is rescued by the introgression of the entire fourth chromosome of *D. arizonae*. In these males no bundle with 64 spermatozoa was observed among 32 examined, and the mean number of mature sperm per bundle was only 57. In addition, the range of this number was much higher than in the other three genotypes and there was clear evidence of a systematic interindividual variation. These differences between $mojY^aSMF^a$ and $mojY^aIV^{a/m}$ suggest that the fourth chromosome contains additional factors that are necessary for full fertility, yet they have no effect on the Y^a/SMF^m incompatibility whose result is unconditional sterility.

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