A Genetic Test to Determine the Origin of Maternal Transmission Ratio Distortion: Meiotic Drive at the Mouse *Om* Locus

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ABSTRACT

We have shown previously that the progeny of crosses between heterozygous females and C57BL/6 males show transmission ratio distortion at the *Om* locus on mouse chromosome *11*. This result has been replicated in several independent experiments. Here we show that the distortion maps to a single locus on chromosome *11*, closely linked to *Om*, and that gene conversion is not implicated in the origin of this phenomenon. To further investigate the origin of the transmission ratio distortion we generated a test using the well-known effect of recombination on maternal meiotic drive. The genetic test presented here discriminates between unequal segregation of alleles during meiosis and lethality, based on the analysis of genotype at both the distorted locus and the centromere of the same chromosome. We used this test to determine the cause of the transmission ratio distortion observed at the *Om* locus. Our results indicate that transmission ratio distortion at *Om* is due to unequal segregation of alleles to the polar body at the second meiotic division. Because the presence of segregation distortion at *Om* also depends on the genotype of the sire, our results confirm that the sperm can influence segregation of maternal chromosomes to the second polar body.

TRANSMISSION ratio distortion (TRD), defined as a statistically significant departure from the Mendelian inheritance ratio expected, has been reported in a broad range of organisms. Two systems in which TRD of paternal alleles is observed, *Segregation distorter* in Drosophila and the *t*-haplotype in the mouse, have been the object of study for several decades and both have been characterized to some degree at the molecular level (Silver 1993; Crow 1999; Merill *et al.* 1999). In both of these cases, TRD results from the inability of some classes of sperm to fertilize ova.

TRD of maternal alleles has been described in mammals, including humans (Evans *et al.* 1994; Shaw *et al.* 1995; Chakraborty *et al.* 1996; Rubinsztein and Leggo 1997; Magee and Hughes 1998; Naumova *et al.* 1998; Eaves *et al.* 1999; Vorechovsky *et al.* 1999) and rodents (Canham *et al.* 1970; Gropp and Winking 1981; Thomson 1984; Biddle 1987; Ruvinsky *et al.* 1987; Ceci *et al.* 1989; Agulnik *et al.* 1990; Justice *et al.* 1990; Siracusa *et al.* 1992; European Mouse Backcross Collaborative Group 1994; Johnson *et al.* 1994; Rowe *et al.* 1994; Montagutelli *et al.* 1996; Pardo-Manuel de Villena *et al.* 1996; Shendure *et al.* 1998; de la Casa-Esperon *et al.* 2000), insects (Nur 1977), and in plants such as maize (Rhoades 1942) and Arabidopsis (Vongs *et al.* 1993). In most of these cases the mechanism giving rise to maternal TRD is unknown. In addition to differential viability of some classes of embryos or gametes, maternal TRD may result from unequal segregation of chromosomes to the polar bodies during meiosis (meiotic drive).

We have observed significant and reproducible maternal TRD at the *Om* locus on mouse chromosome *11* (Pardo-Manuel de Villena *et al.* 1996, 1997). This TRD occurs in crosses in which there is also significant postfertilization loss of embryos due to the "DDK syndrome" (Wakasugi 1974; Babinet *et al.* 1990). This embryonic lethal phenotype was first observed when females from the DDK inbred strain were mated to males of many other inbred strains (Tomita 1960; Wakasugi *et al.* 1967; Wakasugi 1973, 1974). The embryos die at the morula to blastocyst stage because of an incompatibility between a cytoplasmic factor of DDK maternal origin and a paternal non-DDK gene (Mann 1986; Renard and Babinet 1986; Babinet *et al.* 1990). Both maternal and paternal genes have been mapped to the Ovum mutant (*Om*) locus on mouse chromosome *11* (Baldacci *et al.* 1992, 1996; Sapienza *et al.* 1992; Cohen-Tannoudji *et al.* 1996; Pardo-Manuel de Villena *et al.* 1997, 1999) but their molecular identities are unknown. Although postfertilization loss and TRD are weakly correlated (Pardo-Manuel de Villena *et al.* 1996), it is unclear whether the two are mechanistically related.

In this article we have used a classical approach, originally derived from examination of TRD in maize

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TABLE 1

Inheritance of maternal alleles at *D11Mit66*

Cross	Maternal allele at *D11Mit66* non-DDK	DDK
1. [(B6 × C3H)F_1 × DDK] × B6	103	174
2. (B6 × DDK)F_1 × B6	178	258
3. (B6-*Pgk1*^{a} × DDK)F_1 × B6	185	305
4. (B6 × DDK)F_1 × (BALB/c-DBA/2)F_1	132	168
Total	598	905

Numbers following each cross (see materials and methods) represent number of individuals inheriting DDK and non-DDK alleles from heterozygous females.

(Rhoades and Dempsey 1966), that compares the degree of TRD observed among offspring carrying parental *vs.* nonparental chromosomes to discriminate between TRD that is the result of postmeiotic *vs.* meiotic events. We have used this test to determine the origin of the maternal TRD at the *Om* locus on mouse chromosome *11* (Pardo-Manuel de Villena *et al.* 1996, 1997). Our results indicate that TRD at *Om* is due to unequal segregation of non-DDK alleles to the polar body at the second meiotic divison (MII), *i.e.*, meiotic drive (Sandler and Novitski 1957). Because the presence of meiotic drive at *Om* depends on the genotype of the sire, we conclude further that the sperm can influence segregation of maternal chromosomes to the second polar body after fertilization in the mouse. Such an influence of the sire on maternal meiotic drive has also been noted in wild-derived mice that carry a large insertion on chromosome *1* (Agulnik *et al.* 1993).

MATERIALS AND METHODS

Mouse crosses: The backcrosses used in this study are listed in Table 1: (1) [(C57BL/6 × C3H)F_1 × DDK] × C57BL/6, 120 of these offspring were described previously (Pardo-Manuel de Villena *et al.* 1997) while 157 represent new data; (2) (C57BL/6 × DDK)F_1 × C57BL/6, 240 of these offspring were reported previously (Pardo-Manuel de Villena *et al.* 1996, 1997) and 196 represent new data; (3) (C57BL/6-*Pgk1*^{a} × DDK)F_1 × C57BL/6, all 490 offspring are described here for the first time; and (4) (C57BL/6 × DDK)F_1 × (DBA/2 × BALB/c)F_1 and (C57BL/6 × DDK)F_1 × (BALB/c × DBA/2)F_1, all 103 and 197 offspring, respectively, are described here for the first time. In all crosses the dam is listed first and the sire second. The C57BL/6 inbred strain was obtained from the Jackson Laboratory (Bar Harbor, ME) and Harlan Sprague Dawley (Indianapolis, IN). Some of the DDK mice were kindly provided by Dr. C. Babinet (Institut Pasteur, Paris). We are especially grateful to the late Dr. V. M. Chapman (Roswell Park Memorial Institute, Buffalo, NY) for the gift of the C57BL/6-*Pgk1*^{a} congenic strain. The fertility characteristics of these crosses have been described previously (Pardo-Manuel de Villena *et al.* 1999).

Genotype determination: DNA extractions from tail biopsies, gel electrophoresis, and autoradiography were performed as described previously (Maniatis *et al.* 1982; Hogan *et al.* 1986). Oligonucleotide primers for all "*D11Mit*" genetic markers (Dietrich *et al.* 1994) were purchased from Research Genetics (Huntsville, AL). Genotypes were determined as suggested by the manufacturer.

Test for a single distorted locus: The test was performed as described previously (Montagutelli *et al.* 1996). Goodness-of-fit (GF), for N loci analyzed, was estimated by

$$\mathrm{GF} = 4\sum_{i=1}^{N} n_i \left[\arcsin\sqrt{K_{i_{\mathrm{obs}}}} - \arcsin\sqrt{K_{i_{\mathrm{exp}}}}\right]^2,$$

where n_i is the number of animals typed for locus i, $K_{i_{\mathrm{obs}}}$ the observed fraction of offspring that inherit DDK alleles (in our case) at locus i, and $K_{i_{\mathrm{exp}}}$ the expected fraction of offspring that receive maternal DDK alleles at the same locus. Note that the formula reported previously (Montagutelli *et al.* 1996) contained an error. The corrected formula, reported here, was provided by Dr. X. Montagutelli (Institut Pasteur, Paris). GF follows a chi-square distribution with N d.f.

Genetic test: The genetic test that we have generated to determine the origin of maternal TRD is based on the fact that postmeiotic and meiotic selection mechanisms differ in whether they can be affected by recombination between the centromere and the locus at which TRD is observed. The effect of recombination between the centromere and the distorted locus in meiotic drive through female meiosis was first described in maize more than 50 years ago (Rhoades 1942). Since that report, a large amount of evidence in support of this effect has accumulated (Rhoades and Vilkomerson 1942; Rhoades 1952; Rhoades and Dempsey 1966) and a mechanism behind this form of meiotic drive has been proposed and partially confirmed (Peacock *et al.* 1981; Dawe and Cande 1996; Yu *et al.* 1997; Kaszas and Birchler 1998).

The effect of recombination on TRD is summarized in Figure 1. Female meiosis, as represented in Figure 1, has been classified on the basis of the haplotypes that could be present in the four potential meiotic products as parental ditype (PD), tetratype (T), and nonparental ditype (NPD; Figure 1). The type of tetrad is determined by the number of crossovers and the number of strands involved (Weinstein 1936). Offspring are classified on the basis of the chromosomal haplotype inherited, defined by genotype at the centromere and the distorted locus, into one of four possible classes, two parental (p_1 and p_2) and two nonparental (n_1 and n_2).

The reason that recombination has an effect on TRD that occurs during meiosis is that selection of one allele, at the expense of the other, may be accomplished only when the products of a meiotic division may differ in the alleles that will be segregated. This possibility is, in turn, dependent on whether the homologous chromosomes or chromatids being compared have undergone a recombination event between the centromere and the locus at which TRD is observed (Rhoades 1952; Rhoades and Dempsey 1966). Because the first meiotic division (MI) separates homologous chromosomes from one another (Figure 1), selection of one allele over the other can only occur if either there has been no recombination event between the centromere and the distorted locus (class 1 in Figure 1) or there have been two recombination events involving either the same two chromatids or all four chromatids (classes 2 and 6, respectively, in Figure 1). Both chromatids must carry the same, favored, allele if there is to be preferential segregation of the homologous chromosome carrying disfavored alleles to the first polar body. Conversely, each chromatid must carry a different allele if selection is to occur at MII. Because MII separates chromatids (Figure 1), one allele can be segregated preferentially to the second polar body only if the two chromatids carry different alleles. This will be the case only if a recombination event has occurred between the centromere and the distorted locus such that the two chromatids carry different alleles (classes 3,

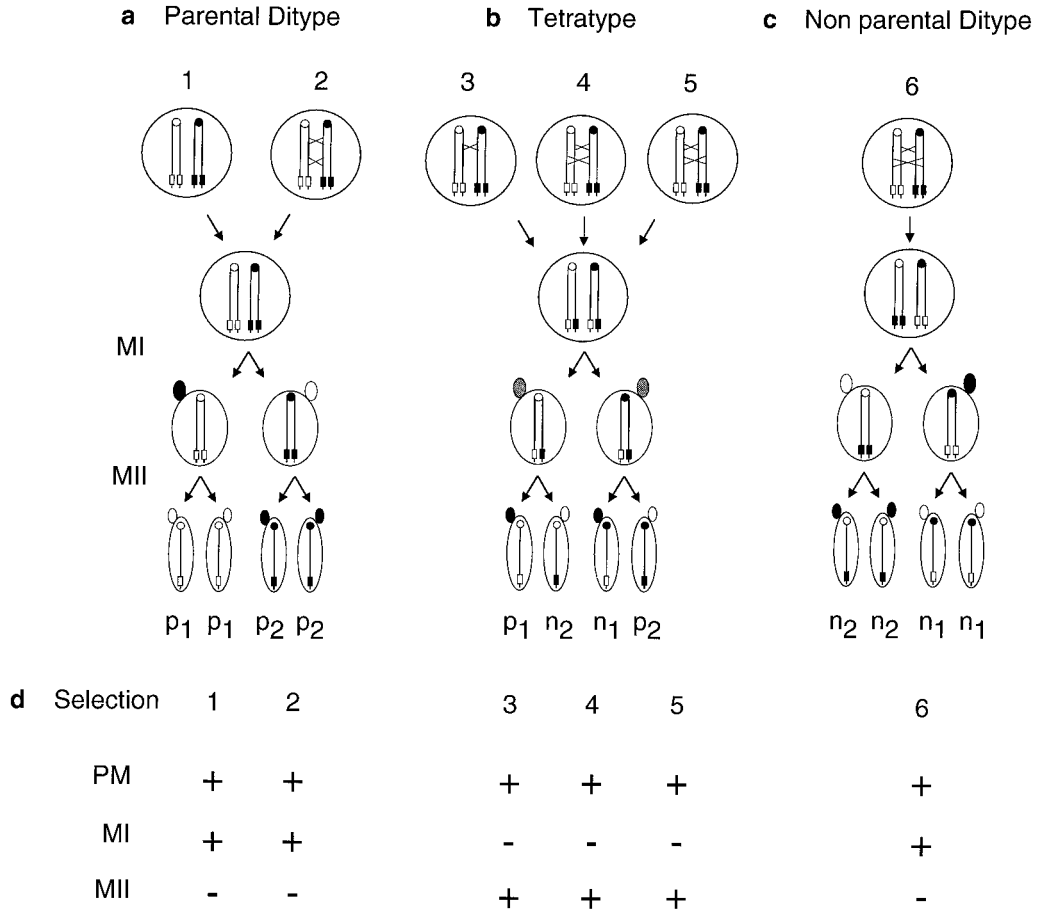

Figure 1.—Classification of ova into tetrads. Although it is not possible to directly determine all of the products of a single meiosis in mammals, the four potential products of any meiosis may be inferred from the single meiotic product available for analysis (Weinstein 1936). When the genotypes are determined at the centromere and the distorted locus, meiosis can be classified on the basis of the haplotypes that could be present in the four potential meiotic products as (a) parental ditype (PD), (b) tetratype (T), and (c) nonparental ditype (NPD). The centromere is represented as an open or filled circle and the locus at which TRD is found by an open or filled rectangle. Open and filled patterns represent different alleles at these two loci. The chromosomal region distal to the distorted locus is not represented, as crossing over in this region is irrelevant for the model. Polar bodies are represented as small ovals outside the meiotic products. The shade of the polar body reflects the presence of the preferentially transmitted allele at the distorted locus (white), the absence of the preferentially transmitted allele at the distorted locus (black), or the presence of both alleles (gray) within the polar body. (a) In PD, two of the potential meiotic products inherit one parental haplotype (p_1), while the other two potential products inherit the other parental haplotype (p_2). (b) T results in four different potential meiotic products, two reciprocal parental (p_1 and p_2) and two reciprocal nonparental (n_1 and n_2). (c) In NPD, two potential products are nonparental of one type (n_1) and two carry the reciprocal nonparental combination of alleles (n_2). (d) Schematic representation of which classes (1–6) are subject to selection when selection is postmeiotic selection (PM), selection at the first meiotic division (MI); or selection at the second meiotic division (MII). +, selection; −, no selection.

4, and 5 in Figure 1). On the other hand, TRD resulting from postmeiotic selection is based on preferential loss of embryos of specific genotype at the distorted locus and is predicted to be independent of the recombinational status of the chromosome carrying the distorted locus. Distinguishing between postmeiotic and meiotic mechanisms of TRD may thus be accomplished by determining whether TRD is independent of the chromosome haplotype inherited by the offspring. Note that the test assumes that TRD has a single origin, postmeiotic, MI, or MII. If the origin of TRD is not homogeneous, the results of the test could be difficult to interpret.

The proper use of this test involves three consecutive steps: First, the system under study must fulfill the following requirements: (i) the TRD should be reproducible and not simply a result of sampling fluctuations; (ii) the TRD should be the result of a single locus (or closely linked loci) on the chromosome in question; (iii) TRD should not result from gene conversion at the distorted locus; and (iv) the locus at which distortion is observed should be linked to the centromere (*i.e.*, significantly <0.5 recombination fraction). We use the term "distorted locus" to designate the locus at which TRD is observed.

Second, if these requirements are fulfilled, the null hypothesis that TRD is the result of postmeiotic selection (*i.e.*, preferential loss of embryos or offspring of a particular genotypic class) is tested by determining whether the level of TRD observed is independent of the chromosome haplotype inherited. Note that failure to reject the null hypothesis could be due to true postmeiotic selection, insufficient power of the data set, or equal selection at both MI and MII.

Third, if the null hypothesis is rejected, the alternative hypothesis, that TRD is the result of meiotic selection, is accepted (given sufficient power). The meiotic origin of the observed TRD (MI or MII) is then determined under the model (Figure 1). MI selection leads to greater TRD among individuals inheriting parental haplotypes than individuals inheriting nonparental haplotypes ($TRD^p > TRD^n$; Figure 1) because classes that are subject to selection (classes 1, 2, and 6) generate more offspring with parental haplotypes than nonparental haplotypes, *i.e.*, selection will affect a larger number of offspring with parental haplotypes. Moreover, classes that are not subject to selection (classes 3, 4, and 5) produce equal numbers of parental and nonparental haplotypes. In contrast, MII selection leads to greater TRD among individuals inheriting nonparental haplotypes than individuals inheriting parental haplotypes ($TRD^n > TRD^p$; Figure 1) because classes that are subject to selection (classes 3, 4, and 5) produce equal numbers of parental and nonparental haplotypes, while classes

that are not subject to selection (classes 1, 2, and 6) produce fewer offspring with nonparental haplotypes than parental haplotypes. We consider that a statistically significant result for rejecting the null hypothesis is obtained when the 95% confidence intervals for TRD^n and TRD^p do not overlap.

These simple qualitative predictions are unlikely to be adversely affected by the second assumption of the model, that of no chromatid interference, unless there is a very high degree of positive chromatid interference. Positive chromatid interference occurs when recombination between two (nonsister) chromatids increases the probability of a crossover on the remaining chromatids (Bailey 1961). This will have the overall effect of increasing the fraction of four-strand doubles (class 6) at the expense of two-strand doubles (class 2; Bailey 1961). To affect the ability to detect meiotic selection, positive chromatid interference must be of such magnitude that the number of offspring from class 6 becomes more than half the number of offspring from the sum of classes 3, 4, and 5 (*i.e.*, the number of nonparental haplotypes from class 6 becomes greater than the number of nonparental haplotypes from the sum of classes 3, 4, and 5). Because nonparental chromosomes are selected at MI in class 6, rather than at MII as in classes 3, 4, and 5 (Figure 1), this will have the effect of decreasing the ability to detect any difference between TRD^p and TRD^n. This circumstance is extremely unlikely; note that class 3 is the sum of all single crossovers, which is expected to be larger than the sum of all other recombinant classes, taken together. Negative chromatid interference [increasing the fraction of two-strand doubles (class 2) at the expense of four-strand doubles (class 6)], on the other hand, will enhance the difference between TRD^p and TRD^n, making meiotic selection at MI or MII easier to detect, because the only confounding class (class 6) will be reduced. Note that there is little consensus on the occurrence of chromatid interference, but available studies seem to argue for an excess of two-strand doubles (Mortimer and Fogel 1974; Zhao *et al.* 1995), *i.e.*, negative chromatid interference (we also present evidence for a low level of negative chromatid interference in our data set; see results). Neither positive nor negative chromatid interference has an effect on the level of TRD resulting from postmeiotic selection under the requirements of the model and therefore does not affect the test under the null hypothesis that TRD originates from a postmeiotic selection.

It is also possible to derive quantitative predictions from this model for the level of TRD that will occur on nonparental haplotypes as a result of meiotic selection at MII (see appendix). However, the precise values obtained are sensitive to the precise level of chromatid interference present. The observed level of TRD will be higher than predicted if there is negative chromatid interference and lower than predicted if there is positive chromatid interference.

Last, it is important to note that the genetic distance (the recombination fraction) between the centromere and the distorted locus establishes the upper limit for the level of TRD that can be observed when TRD is the consequence of maternal meiotic drive. The maximum level of TRD that can be observed by selection at MI decreases as a function of the distance from the centromere, while the maximum level of TRD occuring at MII that can be observed increases as a function of the distance to the centromere, reaching a maximum at 50% of recombination. In contrast, postmeiotic selection is independent of the position of the distorted locus with respect to the centromere, and any level of TRD is possible at any location along a chromosome.

TRD that is the result of maternal meiotic drive rarely exceeds 75% (Agulnik *et al.* 1990; Dawe and Cande 1996); therefore, when TRD is very high (>80%) it is usefull to determine whether the level of TRD observed and the location of the distorted locus are compatible with meiotic drive. An estimate of the maximum level of TRD that can be observed through meiotic drive can be obtained by dividing the offspring into two classes: (i) those arising from achiasmate bivalents (class 1); and (ii) those arising from single crossovers (class 3) and assuming, for this purpose only, that double crossovers do not occur. Note that under these assumptions meiotic drive at MI will select offspring from class 1 but not class 3, while the reciprocal situation will apply to MII selection. The number of offspring in each class can be derived from the dataset as follows: class 3 offspring should be equal to twice the sum of nonparental individuals observed [class $3 = 2(n_1 + n_2)$] because this class generates as many parental as nonparental haplotypes. The remainder of the offspring can be grouped into class 1, under the assumption of no double crossovers [class $1 = (p_1 + p_2 + n_1 + n_2) - 2(n_1 + n_2)$]. The maximum levels of TRD^p and TRD^n possible are then calculated assuming 100% selection in one class and no selection in the other.

RESULTS

TRD on chromosome *11* is reproducible: We have reported TRD at loci linked to the *Om* locus (position 47 cM; Montgomery *et al.* 1998) on mouse chromosome *11* in offspring from heterozygous females (Pardo-Manuel de Villena *et al.* 1996) and have shown that TRD is a reproducible event in three independent backcrosses (Pardo-Manuel de Villena *et al.* 1997). Here we extend these observations by determining the genotype of additional offspring from the same backcrosses at *D11Mit66* (crosses 1 and 2 in Table 1) and by adding two unreported crosses (crosses 3 and 4 in Table 1). TRD in favor of DDK alleles was observed in each of the additional experiments (Table 1). Overall, the level of significance of this observation is very high ($\chi^2 = 62.71$; $P < 10^{-6}$) despite the modest level of overall TRD observed (TRD = 60.2%). We conclude that TRD at *Om* is a constant feature of these crosses and not a sampling effect.

Mapping TRD on chromosome *11*: The fulfillment of the second and third requirements (materials and methods) for using this method for determining the origin of TRD can be demonstrated by analyzing the chromosome *11* haplotypes of the progeny. The genotypes of 457 offspring were determined at 10 loci spanning the entire length of chromosome *11*. The percentage of DDK alleles observed at each locus is shown in Figure 2. In Figure 2a, loci have been placed at the published map locations (Montgomery *et al.* 1998), while in Figure 2b, each locus has been placed at the observed recombination distance from *D11Mit66*. An epistatic interaction between the distorted locus and any other locus (or loci) along chromosome *11* will result in a second peak and/or significant changes in the expected genetic distance. Inspection of Figure 2a shows that TRD has a single maximum in the vicinity of *D11Mit66* and that TRD decreases both proximal and distal as a function of the genetic distance [as given by

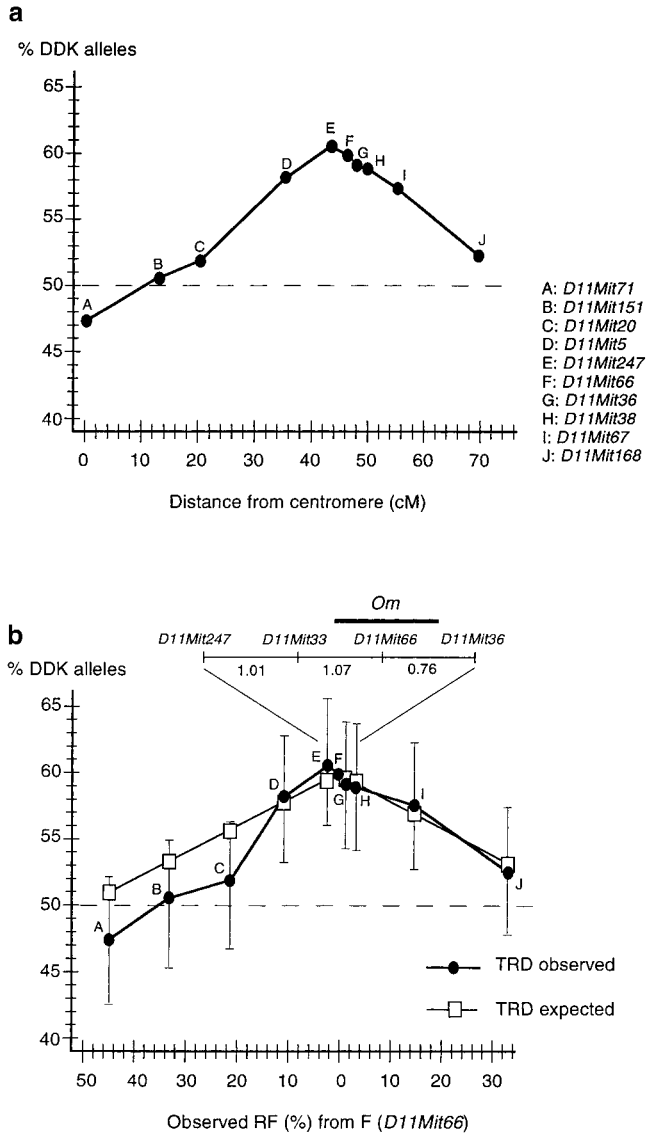

Figure 2.—Segregation of maternal alleles on chromosome *11*. (a) Percentage of DDK alleles observed at each locus on chromosome *11*. Distances from the centromere are as published (Montgomery *et al.* 1998). (b) Comparison between TRD on chromosome *11* with that expected from a single-locus model. Circles denote the observed TRD at each locus with error bars showing the 95% confidence interval. Open squares denote the TRD expected from a model involving a single distorted locus placed at *D11Mit66* with 60.0% of DDK alleles at this locus. The horizontal axis shows the observed recombination fraction (in percentage) between each locus and *D11Mit66*. The inset shows the observed recombination fraction (in percentage) between loci closely linked to *Om* in over 1500 meioses.

the Chromosome *11* Committee Report (Montgomery *et al.* 1998)] from this locus.

A method to determine the number and location of loci involved in TRD has been reported previously (Montagutelli *et al.* 1996). We have used this approach to demonstrate that the observed allele frequency at loci along chromosome *11* is in good agreement with the predictions of the model for a single distorter placed at *D11Mit66* with an expected TRD of 60% (GF = 6.50, 10 d.f., not significant at $P = 0.25$), using the recombination distance observed in our experiment (Figure 2b). Because the object of performing this test was to determine whether TRD can be explained as the result of selection at a single locus, we did not try to map the location of minimum GF more precisely. However, incrementally changing both the location and the expected level of TRD in the vicinity of *D11Mit66* has little effect on the GF (data not shown).

Comparison of Figure 2a and Figure 2b indicates that the location of, and the distances between, the loci characterized in our study are as expected from the reported map locations (Montgomery *et al.* 1998). We conclude that recombination fraction is not significantly altered, ruling out both the presence of inversions and additional distortion controlling loci on chromosome *11*. Moreover, no inversions are detectable in the region spanning *D11Mit66* because we are able to observe recombination between loci that are very closely linked to this locus (Figure 2b). We may also dismiss any significant effect of gene conversion as we find no evidence for chromosomes that are apparent double recombinants, in the subcentimorgan range, at *Om* (based on determining the genotypes of >1500 offspring at *D11Mit247*, *D11Mit66*, and *D11Mit36*). Last, evidence for linkage between the centromere (*D11Mit71*) and the distorted locus (*D11Mit66*) can be obtained from Figure 2b and also from Table 2, in which the number of offspring inheriting nonparental haplotypes is shown to be significantly smaller than the number of offspring inheriting parental haplotypes ($n_1 + n_2 = 664 < 839 = p_1 + p_2$; $\chi^2 = 20.38$; $P < 0.0001$). In addition, the ratio of nonparental:parental haplotypes observed in these crosses is the same as the ratio observed in the $F_1 \times$ DDK backcross, 163:205 (a viable backcross; Sapienza *et al.* 1992; Pardo-Manuel de Villena *et al.* 1999), and the DDK $\times$ F_1 backcross, 83:103 (a semilethal backcross; Sapienza *et al.* 1992). This indicates that the genetic distance is similar in all of these crosses and is not related to the presence of TRD or the viability of the cross. We conclude that TRD at *Om* fulfills all four of the requirements outlined above and that the null hypothesis of a postmeiotic origin of TRD can be tested.

We have also obtained data supporting the model assumption of no positive chromatid interference (see materials and methods) through the examination of the chromosome *11* haplotypes defined by *D11Mit71*, *D11Mit151*, *D11Mit20*, *D11Mit5*, *D11Mit247*, and *D11Mit66*, among 457 offspring (Figure 2b). These are distributed as follows: 229 nonrecombinant chromosomes, 200 single recombinants, 26 doubles, and 2 triples. The proportion of doubles detected denotes a very low level of total interference (both chiasma and chromatid). Because the map distances are in agree-

TABLE 2

TRD at *Om* among offspring inheriting parental and nonparental maternal haplotypes

Cross	Haplotype p_2	p_1	n_2	n_1	TRD^t	TRD^p / TRD^n
1. [(B6 × C3H)F_1 × DDK] × B6	68	91	35	83	62.8	57.2/70.3
2. (B6 × DDK)F_1 × B6	108	127	70	131	59.2	54.0/65.2
3. (B6-*Pgk1*^{a} × DDK)F_1 × B6	108	171	77	134	62.2	61.3/63.5
4. (B6 × DDK)F_1 × (BALB/c-DBA/2)F_1	79	87	53	81	56.0	52.4/60.4
Total	363	476	235	429	60.2	56.7/64.6

Haplotypes are assigned by genotype at the centromere (*D11Mit71*) and the distorted locus (*D11Mit66*). In all crosses the dam is listed first and the sire second. In cross 4, both types of reciprocal F_1 hybrid males were used as sire. p_1, parental DDK at both *D11Mit71* and *D11Mit66*; p_2, parental B6 (or C3H in cross 1) at both *D11Mit71* and *D11Mit66*; n_1, nonparental B6 (or C3H in cross 1) at *D11Mit71* and DDK at *D11Mit66*; n_2, nonparental DDK at *D11Mit71* and B6 (or C3H in cross 1) at *D11Mit66*. TRD^t is the percentage of TRD observed among all offspring in each cross; TRD^p / TRD^n are the levels of TRD observed in offspring bearing parental and nonparental haplotypes, respectively. $TRD^p = [p_1/(p_1 + p_2)] \times 100$ and $TRD^n = [n_1/(n_1 + n_2)] \times 100$.

ment with the consensus map (Figure 2), chiasma interference does not appear to be altered, which suggests a modest excess of multiple recombinants involving two strands (*i.e.*, a low level of negative chromatid interference; see materials and methods).

TRD at *Om* is the result of meiotic drive: Because TRD at *Om* is reproducible (Pardo-Manuel de Villena *et al.* 1997 and this article), appears to result from a single locus that is linked to the centromere, and we find no evidence for gene conversion or positive chromatid interference, all of the requirements for proper use of the genetic test for the origin of TRD have been fullfilled. We may, therefore, test the null hypothesis that TRD is the result of postmeiotic selection of embryos. Under the null hypothesis, selection occurs only as a result of an individual's genotype and is independent of whether that genotype occurs on a parental or nonparental chromosome *11* haplotype. We tested this hypothesis using the 1503 offspring of DDK heterozygous females summarized in Table 2. Each individual was assigned to one of the four maternal chromosome *11* haplotypes (parental or nonparental, carrying a DDK allele at the distorted locus and parental or nonparental, carrying a non-DDK allele at the distorted locus) by determining their genotype at *D11Mit71* and *D11Mit66* (Table 2).

A chi-square test for independence of maternal chromosome *11* haplotype and TRD at *Om* was performed using the sum of the data from all four crosses and the null hypothesis is rejected ($\chi^2 = 9.59$, 1 d.f., $P < 0.0025$). Note that TRD^n (64.6%; 61.0–68.2, 95% confidence interval) is significantly greater than TRD^p (56.7%; 53.4–60.1, 95% confidence interval). Note also that the qualitative result that TRD^n is greater than TRD^p is the same in all four experiments (Table 2). This observation is consistent with the expectations of the model (Figure 1) that TRD occurs at MII.

A further observation that is consistent with meiotic drive as a result of selection at MII may be obtained from the observation that TRD depends on the type of sperm used to fertilize the ova. We have demonstrated that TRD at *Om* in offspring of F_1 females is present when these females are mated to B6 males but not when they are mated to DDK males (Pardo-Manuel de Villena *et al.* 1997; since that report we have generated additional offspring from F_1 females × DDK males that confirm that TRD at *Om* is absent in these crosses—a total of 269 offspring inherit DDK alleles while 281 offspring inherit B6 alleles). Because fertilization in most mammals (including human and mouse; Hamand Veomett 1980) takes place after the completion of MI but before MII, an effect of the sperm on meiosis is most consistent with an effect on MII, rather than MI.

Additional evidence for an effect of both chromosome haplotype in MII segregation and an influence of the genotype of the sire in the drive system is provided in a companion article (Pardo-Manuel de Villena *et al.* 2000)

DISCUSSION

We have observed reproducible TRD of maternal alleles in the vicinity of the *Om* locus on mouse chromosome *11* (Pardo-Manuel de Villena *et al.* 1996, 1997; this report). We have formulated a genetic test, derived from methods of tetrad analysis from single spore data (Weinstein 1936) and analysis of meiotic drive in maize (Rhoades 1942, 1952; Rhoades and Dempsey 1966), to determine whether the TRD observed at *Om* occurs as a result of postmeiotic or meiotic selection. Our analysis of the chromosome *11* haplotypes inherited by >1500 offspring of F_1 females indicates that TRD is the result of preferential segregation of non-DDK alleles to the polar body at MII, *i.e.*, meiotic drive.

Although there are several examples of maternal meiotic drive involving Robertsonian translocations (Gropp and Winking 1981; Ruvinsky *et al.* 1987), only a single prior example of maternal TRD in mammals has been shown to result from meiotic drive in the absence of such chromosomal rearrangement (Agulnik *et al.* 1990). In this case, meiotic drive occurs in females that are heterozygous for HSR (homogeneously staining regions) inserts on mouse chromosome *1* as a result of unequal segregation of chromatids that have recombined between the centromere and the HSR inserts. MII meiotic drive was demonstrated by direct observation of chromosomes during meiosis, because the chromosome involved carried a distinguishing cytological feature, and was confirmed by determining the genotypes of preimplantation embryos (Agulnik *et al.* 1990).

When the mouse chromosome *1* phenomenon was first described, these observations generated some controversy on the prevalence and significance of such meiotic drive systems (Pomiankowski and Hurst 1993; Ruvinsky 1995). Given the fact that we have also demonstrated meiotic drive on chromosome *11* as a result of selection at MII, it is possible that such systems are not rare. The mechanism by which preferential segregation to the polar body might be achieved is unclear, but could be similar to the mechanism described in maize. Meiotic drive observed in maize (Rhoades 1942) appears to result from a "neocentromeric" activity that is conferred on normally quiescent heterochromatic "knobs" by the presence of a mutation at the *suppressor of meiotic drive* (*smd1*) locus carried by a variant chromosome *10* (Rhoades 1942, 1952; Rhoades and Dempsey 1966; Dawe and Cande 1996). These knobs exhibit differential interaction with the meiotic spindle such that chromosomes carrying the knobs are preferentially segregated to the basal cell that develops into the megagametophyte (Dawe and Cande 1996; Yu *et al.* 1997). Mouse oocytes are not known or thought to possess any maize-like physical polarity that might determine which meiotic product becomes the ovum, as opposed to one of the polar bodies. However, the meiotic spindle at MII is not oriented prior to fertilization in the mouse (Hogan *et al.* 1986). Any process that influenced the orientation of the spindle at MII, based on the presence of a chromosomal feature such a neocentromere, could also result in the preferential segregation of the Om^{DDK} allele to the ovum and/or the Om^{B6} allele to the polar body.

Two of the most-well-studied examples of TRD (meiotic drive), *Segregation distorter* in Drosophila and the *t*-haplotype in the mouse, originate during gametogenesis in males. In these cases, TRD results from the inability of some classes of sperm to fertilize ova (Silver 1993; Crow 1999; Merill *et al.* 1999). Note that the nature of the maternal meiotic drive we are describing on chromosome *11* and that described by Agulnik and coworkers (1990) on chromosome *1* is distinct from such systems. Female gametogenesis in mammals results in only a single functional product of meiosis, the ovum. Meiotic drive in these systems results from the unequal segregation of a chromosome/chromatid to the single functional product of meiosis. In contrast, all four meiotic products of male gametogenesis become sperm. In the absence of gene conversion at a particular locus, it is not possible to create more gametes of one type than another except by reducing the viability or functionality of some sperm. As noted by Ganetztky (1999), such cases do not conform to the original definition of meiotic drive (Sandler and Novitski 1957). If TRD is observed through males, it must reflect differential viability or functionality of some classes of male gametes or embryos.

A strong influence of the genotype of the sire has been demonstrated in instances of maternal TRD (Agulnik *et al.* 1993; Montagutelli *et al.* 1996; Pardo-Manuel de Villena *et al.* 1997; de la Casa-Esperon *et al.* 2000). In all of these cases, TRD is observed among the offspring when they have been sired by males of one genotype but not when sired by males of another genotype. Because TRD on chromosome *X* results from postfertilization loss of embryos (Montagutelli *et al.* 1996; de la Casa-Esperon *et al.* 2000), the effect of the genotype of the sire is readily explained as due to the death of embryos that inherit lethal genotypic combinations of maternal and paternal genes. More puzzling are the observations that TRD on chromosomes *1* and *11* depends on the genotype of the sire. In these cases TRD occurs at the second meiotic division of the ovum, which does not occur until after fertilization in the mouse (Ham and Veomett 1980). The influence of the genotype of the sire indicates that the sperm may affect the segregation of maternal chromatids to the second polar body. Whether the segregation of chromatids at MII is generally influenced by the sperm or is a response of these particular ova to particular genotypic classes of sperm is unclear. However, the sperm genome is present within the ovum for up to several hours before second polar body extrusion takes place (Hogan *et al.* 1986), so there is ample opportunity for the implementation of whatever pathway is involved. The existence of such a mechanism has potentially important implications in evolutionary biology (Pomiankowski and Hurst 1993) and for the widespread use of some types of assisted reproductive technology.

Finally, we point out the utility of the genetic test described in this article to investigate the origin of maternal TRD in a number of instances in the human (Evans *et al.* 1994; Shaw *et al.* 1995; Chakraborty *et al.* 1996; Rubinsztein and Leggo 1997; Magee and Hughes 1998; Naumova *et al.* 1998; Eaves *et al.* 1999; Vorechovsky *et al.* 1999). The origin of maternal TRD in humans has always been controversial because direct tests of meiotic drive *vs.* postfertilization death are not

possible. The test that we propose here is straightforward and of general applicability. It may be used to obtain preliminary, and in some cases definitive, information on the nature of the selection operating in a given system. In two companion articles we show its use to determine the origin of other cases of maternal TRD (de la Casa-Esperon *et al.* 2000; Pardo-Manuel de Villena *et al.* 2000).

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APPENDIX

It is possible to estimate the expected values of p_1, p_2, n_1, and n_2 in the case of meiotic drive at MII under the assumption of no chromatid interference. In this situation the expected proportion of each of the four double recombinant classes (classes 2, 4, 5, and 6 in Figure 1) is the same. Overall, classes with at least one recombination event (classes 2–6, Figure 1) produce equal numbers of offspring carrying parental and nonparental chromosomes. Classes 3–5 each produce two parental and two nonparental products, while classes 2 and 6, combined, produce four parental and four nonparental products. Because the sum of p_1 haplotypes equals the sum of n_1 haplotypes and the sum of p_2 haplotypes equals the sum of n_2 haplotypes among the combined classes 2–6 (Figure 1), the level of TRD in parental *vs.* nonparental haplotypes arising from these classes is predicted to be the same. Therefore the estimate for the total number of offspring arising from classes with at least one recombination event is

$$2(n_1 + n_2). \tag{A1}$$

However, achiasmate bivalents produce only parental haplotypes (class 1, Figure 1). The number of parental chromosomes arising from class 1 may be estimated directly from any dataset by subtracting twice the number of nonparental chromosomes observed from the total. Note that because the meiotic products from the sum of the recombinant classes produce exactly as many parental chromosomes as nonparental chromosomes, twice the number of nonparental chromosomes must be subtracted (Equation A1) from the total to obtain the number of parental chromosomes arising from achiasmate bivalents. Then, the estimated number of offspring arising from class 1

$$N - 2(n_1 + n_2), \tag{A2}$$

where N is the total number of offspring. The number of offspring in class 1 carrying the favored allele is expected to be one-half of (A2) because there is no MII selection in class 1:

$$0.5[N - 2(n_1 + n_2)]. \tag{A3}$$

The total number of offspring carrying the favored allele is

$$(p_1 + n_1). \tag{A4}$$

Therefore, the number of offspring carrying the favored allele arising from recombinant classes (classes 2–6) should be (A4) − (A3):

$$(p_1 + n_1) - 0.5[N - 2(n_1 + n_2)]. \tag{A5}$$

The expected level of TRD in classes 2–6 can be determined by dividing the previous value by the total number of offspring arising from these classes (A1). There-

fore, the expected level of TRD in nonparental haplotypes is

$$E(\mathrm{TRD}^{n}) = \{(p_1 + n_1) - 0.5[N - 2(n_1 + n_2)]\}/2(n_1 + n_2).$$

In our experiment, the expected level of TRD^{n}, under the assumption of no chromatid interference, is 61.6%. This level of TRD^{n} is consistent with the observed level of 64.6% (61.0–68.2, 95% confidence interval). The difference between the expected and observed level of TRD^{n} probably reflects the presence of a low level of negative chromatid interference, which is predicted to result in an observed level of TRD^{n} that is higher than the expected.