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Nondisjunction Rates and Abnormal Embryonic Development in a Mouse Cross Between Heterozygotes Carrying a (*7, 18*) Robertsonian Translocation Chromosome

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ABSTRACT

Mice bearing Robertsonian translocation chromosomes frequently produce aneuploid gametes. They are therefore excellent tools for studying nondisjunction in mammals. Genotypic analysis of embryos from a mouse cross between two different strains of mice carrying a (*7, 18*) Robertsonian chromosome enabled us to measure the rate of nondisjunction for chromosomes *7* and *18*. Embryos (429) were harvested from 76 litters of mice and the parental origin of each chromosome *7* and *18* determined. Genotyping these embryos has allowed us to conclude the following: (1) there were 96 embryos in which at least one nondisjunction event had taken place; (2) the rate of maternal nondisjunction was greater than paternal nondisjunction for the chromosomes sampled in these mice; (3) a bias against chromosome *7* and *18* nullisomic gametes was observed, reflected in a smaller than expected number of uniparental disomic embryos; (4) nondisjunction events did not seem to occur at random throughout the 76 mouse litters, but were clustered into fewer than would be expected by chance; and (5) a deficiency of paternal chromosome *18* uniparental disomic embryos was observed along with a higher than normal rate of developmental retardation at 8.5 days post coitum, raising the possibility that this chromosome has at least one imprinted gene.

AUTOSOMAL aneuploidy in mammals adversely affects developmental processes. It has been estimated that 0.3% of human newborns are aneuploid (HASSOLD 1985). In humans, >70% of the chromosomal errors associated with early spontaneous abortion and almost half of those detected among newborns involve aneuploidy (BOND and CHANDLEY 1983). The most common cause of genetic mental retardation in humans is due to trisomy of chromosome *21* (GEARHART *et al.* 1987) with 2–3% of trisomy *21* cases resulting from Robertsonian translocation chromosomes (GARDNER and SUTHERLAND 1989). The most likely explanation for the profound phenotypic effects resulting from aneuploidy of whole or parts of a chromosome is an imbalance in gene dosage.

The mouse offers several advantages for studying chromosomal aberrations such as aneuploidy. Many mouse strains exist that carry Robertsonian translocation chromosomes that have been maintained in inbred strains allowing studies to be performed in a defined genetic background. Robertsonian chromosomes in mice are the result of a centric fusion between two chromosomes, producing a single metacentric chromosome without apparent loss of genes, and displaying no phenotypic abnormalities. Robertsonian chromosomes segregate normally when present in a homozygous state in mice, but experience a higher than normal frequency of nondisjunction when heterozygous. Complementation of unbalanced gametes produced by intercrossing mice carrying Robertsonian chromosomes has been used extensively to study the frequency and effects of nondisjunction events (SNELL 1946; SEARLE *et al.* 1971; LYON *et al.* 1976; SEARLE and BEECHEY 1978, 1982) where embryo lethality or phenotypic markers were used to analyze the products of nondisjunction.

In this study, highly polymorphic molecular markers were used to distinguish five strains of inbred mice from one another (Figure 1). Mice carrying a (*7, 18*) Robertsonian chromosome were used to determine the outcomes of meiosis under conditions of elevated rates of nondisjunction. Two genetically distinguishable strains of mice, each with two copies of a (*7, 18*) Robertsonian chromosome were crossed to a second strain with no Robertsonian chromosome (Figure 2, top). Mice in the F_1 generation were phenotypically normal, but carried one copy each of the (*7, 18*) Robertsonian chromosome and one normal chromosome *7* and *18*. These F_1 mice were intercrossed (for example, Figure 2, middle) and chromosomes *7* and *18* typed in the resulting F_2 progeny, harvested before birth. Several different combina-

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Locus	Size in bp				
	B	R	C	D	3
D7MIT25	110	88	144	108	96
D7MIT222	147	155	178	123	123
D7MIT52	164	170	146	160	162
D18MIT14	108	112	130	103	107
D18MIT110	149	110	125	153	117
D18MIT87	144	130	176	152	140
D8MIT4	175	180	191	195	195
D2MIT32	102	107	98	110	102

R is *Rb(2,18)2Lub(7,18)9Lub*
D is DBA/2J
B is C57BL/6JEi-*Rb(7,18)9Lub*
C is *Mus castaneus*
3 is C3H/HeJ

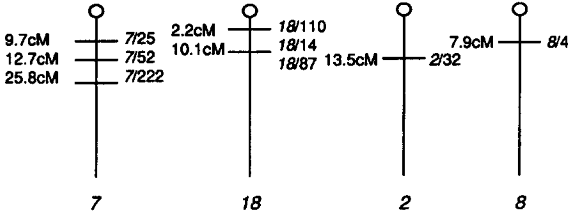

FIGURE 1.—Locus, fragment size and chromosomal location of the polymorphic markers used to genotype F_2 embryos.

tions of parental strains were crossed to produce the embryos (Table 1, first column). Because all of the parental strains could be distinguished by molecular methods, the parental origin and numbers of copies of chromosome *7* and *18* were determined for each F_2 offspring. The phenotype (normal or retarded development at the day of harvest) of each embryo was then correlated with its chromosome *7* and *18* genotype (normal, trisomic or uniparental disomic).

MATERIALS AND METHODS

Mice: All mouse strains were obtained from The Jackson Laboratory. *Rb(2, 8)2Lub(7, 18)9Lub* and C57BL/6JEi-*Rb(7, 18)9Lub* strains were obtained from the Robertsonian Resource at The Jackson Laboratory. *Rb(2, 8)2Lub(7, 18)9Lub* has been maintained on its own inbred background, a combination of ~50% wild-derived *Mus m. domesticus* and 50% laboratory mouse strain background (DAVISSON and AKESON 1993). The second Robertsonian mouse was derived by repeated backcrossing to a C57BL/6J-Ei background. *Rb(2, 8)2Lub(7, 18)9Lub* was crossed to DBA/2J and C57BL/6JEi-*Rb(7, 18)9Lub* was crossed to either *M. castaneus* or C3H/HeJ to generate mice carrying one copy of the (*7, 18*) Robertsonian chromosome (Figure 2, middle). Male F_1 mice from the C57BL/6JEi-*Rb(7, 18)9Lub* × *M. castaneus* cross were not fertile but male F_1 mice from the reciprocal cross were fertile [therefore C3H/HeJ mice were introduced into the cross to generate F_1 males with a maternally inherited C57BL/6JEi-*Rb(7, 18)* chromosome]. All of the combinations of intercrosses used are detailed in Table 1 (first column). Females were used as generated and not especially selected, but some females were tested more often, because, for example, DBA/2J females had more litters than Castaneus females.

F_1 mice with a single copy of the (*7, 18*) Robertsonian chromosome were intercrossed to produce 429 F_2 progeny that were harvested from 8.5 (349 embryos), 9.5 (23 embryos) and 10.5 (57 embryos) days post coitum (dpc) in timed matings. Immediately after removal from the uterus, the embryos were dissected free from extra embryonic tissue and yolk sac and placed in a drop of PBS. Each embryo was examined under a dissection microscope, and the phenotype scored as either normal or retarded. A normal phenotype was scored

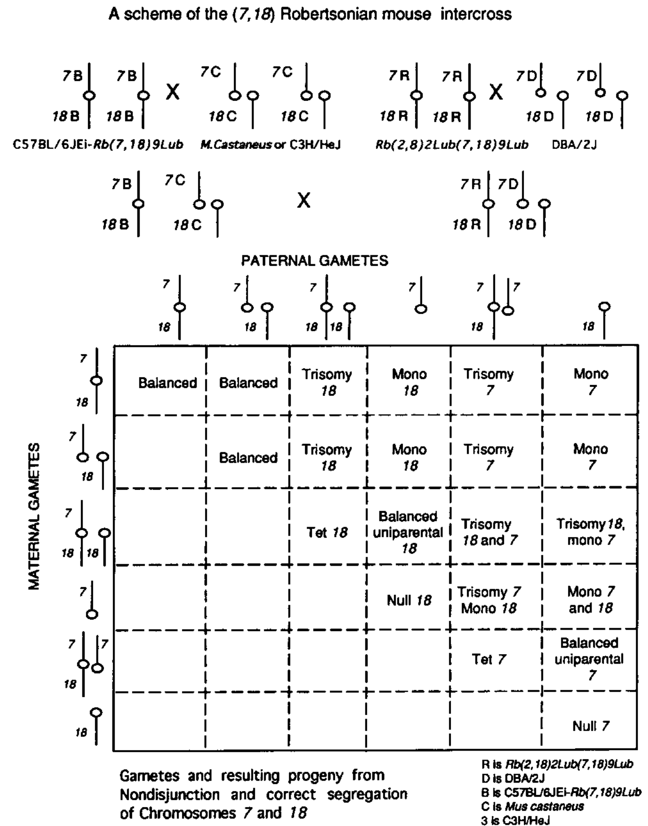

FIGURE 2.—Top shows two of the six parental genotype combinations used to generate the F_1 mice used in this study. The middle shows one of the 10 combinations of F_1 genotypes used to generate the F_2 embryos analyzed in this study. The bottom shows the theoretical outcomes that result from nondisjunction events in the F_1 gametes.

when the embryo appeared to be the correct size and at the appropriate stage of development for its time of harvest. An abnormal phenotype was scored when the embryo either appeared smaller that expected or had a visible developmental problem, as determined by comparison with normal embryos detailed in KAUFMAN's Atlas of Mouse Development (KAUFMAN 1992). After staging, a tissue sample was removed from the tip of the embryo tail and frozen for DNA preparations.

DNA preparation: The embryo tail samples were vortexed in 100 μl of a solution containing 100 mM KCl, 20 mM Tris HCl (pH 8.3), 5 mM $MgCl_2$, 1.4 μM sodium dodecyl sulfate, 40 mM dithiothreitol, 2 mg/ml gelatin and 0.1 mg/ml proteinase K. The samples were incubated at 37° for 1 hr, placed at 95° for 5 min and stored at −20°.

Primers and PCR: Simple sequence repeat (SSR) polymorphism primers (Map Pairs^R) were purchased from Research Genetics, Huntsville, AL. The names of the Map Pairs^R used and the polymorphic bands for each mouse strain along with chromosomal locations are detailed in Figure 1. Genotyping was performed on 1μl samples of DNA using PCR with radioactively labeled primers (DIETRICH *et al.* 1992). The PCR products were visualized on polyacrylamide gels. DNA was amplified in a 10 μl reaction using 1 unit of AmpliTaq DNA polymerase (Perkin Elmer Cetus) with manufacture's buffer in the presence of 0.4 mM dNTPs and both primers at a concentration of 100 mM unlabeled, with one labeled 20 mM primer. Primers were labeled with gamma ^{32}P ATP using T4 polynucleotide kinase (NEB) (TABOR 1994). Amplification conditions were: 3 min at 94° followed by 30 cycles of 94° for

TABLE 1

Parental genotypes of all embryo categories investigated

Parents	Total litters	No nondisjunction	*7* Tri	*18* Tri	*7* UPD	*18* UPD	Double Tri
BXC × DXR	10	2	3	8	1	1	4
BXC × RXD	7	2	3	3	1	1	2
CXB × DXR	0	0	0	0	0	0	0
CXB × RXD	3	1	0	1	0	0	0
BX3 × DXR	3	1	0	2	0	0	0
BX3 × RXD	8	2	3	1	0	2	1
3XB × DXR	0	0	0	0	0	0	0
3XB × RXD	1	1	0	0	0	0	0
DXR × CXB	15	5	10	4	2	6	0
RXD × CXB	5	1	1	3	3	0	0
DXR × BX3	15	6	5	6	2	4	0
RXD × BX3	9	4	6	6	2	0	0
Total	76	22	31	34	11	14	7

The first column lists all of the combinations of parental strains used in the crosses described in the paper. The second column lists the number of litters from each of these crosses. The third column lists the parental origin of litters with no nondisjunction events. The subsequent columns list the parental origins of all of the aneuploid categories.

15 sec, 55° for 30 sec, then 72° for 30 sec, followed by a final 72° for 7 min. PCR products were diluted 1:1 with 100% formamide loading buffer containing bromophenol blue and xylene cyanol, heated at 80° for 2 min, 6 μl was loaded onto an 8% denaturing polyacrylamide gel until the bromophenol blue dye had reached the bottom. Gels were dried, exposed to Kodak X-ray film and the genotypes scored. Each embryo was typed for chromosomes *7* and *18* with one marker polymorphic among the five strains used in this study. To confirm the genotyping for embryos with trisomies or UPD for either chromosome, the embryos were retyped with additional markers. A subset of embryos were also typed for chromosomes *2* and *8*.

Statistical analyses: A number of different statistical tests were employed for analysis of the data.

Chi-squared analyses to determine the distribution of nondisjunction events between mouse litters: The distribution of embryos with nondisjunction events was examined among the 76 litters of mice. The expected number of abnormal embryos per litter was too small for the chi-squared distribution to be a good approximation to that of our chi-squared statistic. The litter size varied from 3 to 10 pups, and direct use of the Poisson distribution of numbers of abnormalities does not take this into account. Therefore two statistical methods were applied to investigate randomness of nondisjunction among litters. In one, we rigorously simulated the null hypothesis of random allocation using a Monte Carlo method. Abnormality, defined as aneuploidy or uniparental disomy, was randomly allocated to each of the 429 embryos, and the resulting number of abnormals were counted in each litter and the chi-squared recomputed. This random reallocation process was repeated 10,000 times and a tally kept of how often the random allocation chi-squared statistic equaled or exceeded that of the data chi-squared statistic. The P value was taken and this tally divided by 10,000.

Maximum Likelihood test for randomness of the distribution of nondisjunction events between mouse litters: The second method applied a likelihood ratio as an additional test of significance. In this test, the null hypothesis was that p was equal to the probability of abnormality being constant over all of the embryos. The alternative hypothesis was that all litters have individual probabilities of abnormality, $p(i)$. Then Generalized Likelihood Ratio (chi-squared) = $-2\log(L_0/L_1)$. Where L_0 was data likelihood under the null hypothesis and L_1 was data likelihood under the alternative. Each likelihood was the product of binomial distributions: $b[a(i), n(i), p(i)]$, where $a(i)$ was the number of abnormal embryos in litter i, $n(i)$ the number of embryos and $p(i)$ the maximum likelihood estimate of the true probability of abnormality under the appropriate hypothesis.

Maximum Likelihood Estimates (MLE) of the rate of normal versus disomic and nullisomic gametes: A generalized likelihood ratio statistic was used to test two hypotheses. In the first, a null hypothesis, that the probability of a normal gamete, $p(n)$, the probability of a disomic gamete, $p(d)$, and the probability of a nullisomic gamete, $p(o)$, are all equal to $^1/_3$, was tested against an alternative hypothesis that the probability of normal, disomic, and nullisomic gametes can assume any value and sum to 1. In the second hypothesis, $p(d)$ and $p(o)$ were assumed equal to each other but not equal to $p(n)$, *vs.* the same alternative hypothesis described above. The assumptions made in generating the likelihood ratio statistics were that there would be no monosomic embryos at or after 8.5 dpc and trisomies, UPDs and normal embryos were equally likely to survive to 8.5–10.5 dpc. The maximum likelihood estimates were made for the data (Table 2) by setting derivatives to 0 and solving and the search method. The data were entered into a computer program designed with the above criteria to calculate the best estimates for the generalized likelihood ratio statistic and P values for each hypothesis.

RESULTS

Incidence of nondisjunction: Embryos harvested at 8.5 (349), 9.5 (23) and 10.5 dpc (57) were scored for the number and parental origin of chromosomes *7* and *18*. The total number of embryos genotyped was 429 and the incidence of chromosomal aneuploidies for *7* and *18* are summarized in Table 2 along with the chromosome parental origins. As expected, there were

TABLE 2

Frequency of various chromosome complements in recovered embryos

Chromosome	No. of embryos	Maternal nondisjunctions	Paternal nondisjunctions
Chromosome *7* Trisomy	31	15	16
Chromosome *18* Trisomy	34	20	14
Chromosomes *7* + *18* Trisomies	7	8	6
Paternal *7* uniparental disomy	4	0	8
Maternal *7* uniparental disomy	7	14	0
Paternal *18* uniparental disomy	2	0	4
Maternal *18* uniparental disomy	12	24	0
Normal complement	330	—	—
Total number of embryos	429	81	48

Number, classification and parental origin of aneuploid embryos identified in 429 individuals.

no monosomic chromosome *7* or *18* embryos detected at 8.5–10.5 dpc, probably because either nullisomic gametes were unlikely to participate in conception or monosomy was lethal in the embryo before 8.5 dpc, or both.

Clustering of nondisjunction events: Table 3 shows the distribution of embryos with nondisjunction events across the 76 litters in the study. Embryos with nondisjunction events did not seem to be randomly distributed among the litters of mice but were clustered into fewer litters than expected. To investigate this observation, statistical analyses were performed to test the hypothesis of random allocation of abnormalities (including trisomic and UPD embryos for chromosomes *7* and *18*) among litters. The value of the chi-squared statistic was calculated. For a sample of 429 embryos, there were 65 trisomies, 25 UPDs and 7 double trisomies distributed amongst the 76 litters (Table 3). The chi-squared statistic calculated for these data is 79.81389 with a P value of 0.009. For the generalized likelihood ratio, the P value is 0.013, similar to that of the chi-squared statistic. Both of these statistical analyses support the observation that nondisjunction events occurring in those gametes that produced aneuploid or UPD embryos were clustered among particular matings and not randomly distributed between the 76 litters ($P = 0.009–0.013$).

Comparison of chromosome aneuploidy and embryo development: The phenotype (normal *vs.* retarded development) was compared with the genotype of chromosomes *7* and *18* for each embryo in the study. The results are summarized in Table 4. The total number of embryos were those that have been phenotyped and genotyped (not including 15 that were not assigned a phenotype).

TABLE 3

Distribution of nondisjunction events among litters

No. nondisjunction events	0	1	2	3	>3
No. of litters	25	13	21	9	8

Distribution of nondisjunction events among 76 litters of mice.

Normal embryos: Among normal genotypes, 18% of embryos were retarded in phenotype at 8.5 dpc, similar to the rate expected in any normal mouse litter.

Trisomic embryos: There were 31 phenotyped embryos with trisomy of chromosome *7* (Table 4). Twenty-seven embryos were found at 8.5 dpc, 10 (37%) had developed normally and 17 (63%) were retarded. There were three at 10.5 dpc, one at 9.5 dpc, each retarded. Embryos with trisomy *7* experience a higher than normal rate of retardation at 8.5 dpc; thirty-four embryos had trisomy *18*, of these, 21 (61%) had developed normally and 13 (38%) were retarded. Of the 34, seven embryos were harvested at 10.5 dpc, two at 9.5 dpc and 25 at 8.5 dpc. Chromosome *18* trisomy was not as severe as chromosome *7* trisomy, because almost twice as many embryos developed normally to 8.5 dpc. Of the seven embryos with trisomy of both chromosomes *7* and *18*, only one out of seven was normally developed (five were found at 10.5 dpc and two at 8.5 dpc).

TABLE 4

Phenotype of embryos with various chromosome complements

Genotype	n	Phenotype: Normal No.	Normal Percent	Retarded No.	Retarded Percent
Normal	333	275	(82)	56	(18)
Trisomy *7*	31	10	(32)	21	(68)
Trisomy *18*	34	21	(61)	13	(38)
Trisomy *7* and *18*	7	1	(14)	6	(86)
UPD *7*	11	3	(27)	8	(72)
UPD *18*	14[a]	7	(54)	6	(46)
Total embryos	429[b]	298	(69)	115	(27)

Comparison of genotype with the phenotype of both normal and aneuploid embryos.

[a] One embryo of unknown phenotype.

[b] Fifteen embryos of unknown phenotype.

Because a second Robertsonian chromosome involving chromosomes *2* and *8* segregated in the Rb(2,8)-2Lub(*7, 18*)9Lub strain of mouse, we questioned whether the retarded phenotype of the trisomy *18* or trisomy *7* embryos was related to their genotype for chromosomes *2* or *8*. In other words, were the more severe trisomy *18* or trisomy *7* phenotypes due to nondisjunction of the (*2, 8*) Robertsonian chromosome? When the embryos with trisomy *18* were genotyped for chromosomes *2* and *8* (data not shown), only one trisomy *18* embryo was also trisomic for chromosome *8* and surprisingly showed a normal phenotype at 8.5 dpc. No trisomy *18* embryos were found to have a trisomy of chromosome *2*. Among a subset of normally developed trisomy *7* embryos, no trisomies of chromosome *2* or *8* were found (data not shown). A single example of a trisomy *7* embryo was found to also have trisomy of chromosome *8* but this embryo had retarded development at 10.5 dpc. Therefore we concluded that aneuploidy for chromosomes *2* or *8* due to the (*2, 8*) Robertsonian chromosome segregating in one of the parental strains of mice could not explain why some trisomy *18* or trisomy *7* mice appeared to be phenotypically normal while others were not. Strain differences also could not be correlated with the degree of normal or retarded developmental phenotype in embryos with trisomy of *7* or *18*.

UPD embryos: Three out of 11 embryos with UPD of chromosome *7* developed normally to 8.5 dpc. The remaining eight were 0.5–1 day retarded. As there were seven maternal UPDs and four paternal UPDs; no obvious difference in the frequency of each parental origin for UPD could be detected. Seven out of 14 embryos with UPD of chromosome *18* developed normally, six were retarded and one was of unknown phenotype; this is a higher rate of retardation than among embryos with a normal genotype. In contrast to the situation with chromosome *7*, only two paternal UPD *18* embryos were detected compared with 12 maternal UPD embryos.

Frequency of maternal and paternal nondisjunction: The frequency of nondisjunction in this mouse cross was 129 events out of a possible 858 gametes. This was calculated based on a trisomy being the product of 1 and a UPD the product of two nondisjunction events. There were 81 out of 858 maternal nondisjunctions and 48 out of 858 paternal nondisjunctions (Table 2, columns 3 and 4). There was a larger number of maternal nondisjunction events than paternal ones. A two-tailed test of binomial proportions showed this to be significant $P = 0.002325$.

Maximum likelihood estimates for nullisomic and disomic gametes: Figure 2 shows the possible outcomes in the embryo from the combinations of gametes generated in the mouse cross in this study. Each embryo (Table 2) is the product of the parental gametes after meiosis. A normal embryo is the product of two normal gametes (containing one copy of chromosome *7* and one of chromosome *18*), a trisomic embryo results from a normal and disomic gamete, and most UPD embryos are the product of two abnormal gametes, a disomic and a nullisomic gamete. Monosomic *7* or *18* embryos would have been the products of one nullisomic and one monosomic gamete but, as expected, none were detected in this protocol. Thus, the number of nullisomic gametes may be inferred only from the numbers of UPD embryos. Although the number of disomic and nullisomic gametes might be theoretically expected to be the same (Figure 2), there appeared to be a smaller than expected number of UPD embryos reflecting a deficit of nullisomic gametes, just as other empirically determined studies have shown. A maximum likelihood estimate of the numbers of disomic compared with nullisomic gametes was performed to investigate this observation.

There were two statistical tests performed: (1) a null hypothesis that monosomic, disomic and nullisomic gametes are equally probable, or $p(o) = p(n) = p(d) = {}^1/_3$, *vs.* the most general test hypothesis that each can assume any value summing to one, or $p(o) + p(n) + p(d) = 1$. For these two hypotheses, the generalized likelihood ratio of the chi-squared statistic with 2 degrees of freedom was 600, where the probability of $\chi^2 > 600 < 10^{-7.}$ (2) The second test had as its null hypothesis that the fraction of gametes with an extra chromosome (disomic) was the same as the fraction of gametes lacking an entire chromosome (nullisomic), or $p(d) = p(o)$, but that neither equaled ${}^1/_3$, *vs.* the same general alternative. For these hypotheses, the generalized likelihood ratio of the chi-squared statistic with 1 degree of freedom was 23 where the probability of the $\chi^2 > 32 = 1.4 \times 10^{-6.}$ Both of these hypotheses were therefore strongly rejected. Based on the data for 429 embryos, the maximum likelihood estimates of the three probabilities $p(n)$, $p(d)$ and $p(o)$ were 0.85, 0.10 and 0.04, respectively. Thus, it is overwhelmingly likely that there is a bias against finding embryos resulting from aneuploid gametes, especially gametes that lack a chromosome.

DISCUSSION

Incidence of nondisjunction: The germ cells of mice carrying a single Robertsonian chromosome undergo a higher than normal frequency of nondisjunction. The resulting gametes would theoretically be predicted to be normal about a third of the time, disomic, a third of the time and nullisomic, a third of the time (Figure 2). Short of genotyping or karyotyping individual gametes, the only way to determine empirically the frequency of aneuploid gametes is to allow fertilization and then infer the gametic genotypes from the resulting embryonic genotypes. Under the assumption that no monosomic embryos would be detected at or after 8.5 dpc, and assuming normal, trisomic, and UPD embryos all develop equally well to at least to 8.5 dpc, we used the incidence of aneuploidy in the embryos to infer the relative frequency of aneuploidy in gametes.

The rate of nondisjunction in this mouse cross is 15%. This is consistent with rates of nondisjunction seen in other Robertsonian crosses. For a (*2, 6*) Robertsonian heterozygote intercross the rate of nondisjunction was reported to be 17% when measured in oocytes (CHEWBOTAR and BARILYAK 1994), whereas a double Robertsonian heterozygote intercross experienced nondisjunction in 28–36% of embryos (BEECHEY and SEARLE 1988). Crosses involving female heterozygotes and male hemizygotes for an *X*:autosome intercross underwent nondisjunction at ~10% in spermatocytes and 5% in offspring (TEASE and FISHER 1991; ADLER *et al.* 1989) although *X*:autosome Robertsonians are not entirely comparable with rearrangements involving autosomes only (TEASE and FISHER 1991). Overall, nondisjunction events during female meiosis I caused more (63%) of the trisomies and UPDs than male meiosis I (37%), implying a gender difference in the incidence of nondisjunction. Of course, nondisjunction events that occurred during meiosis II would not have been detected in this cross. However, because Robertsonian chromosomes are not likely to cause an increase in frequency of nondisjunction of chromatids in meiosis II, we would not expect such events to have distorted these data appreciably. Only limited data have been available for comparing rates of nondisjunction in males *vs.* females experiencing embryonic lethality because most aneuploid embryos have been characterized by karyotypic analysis, however, first cleavage embryos may be sex distinguished because the female chromosomes are slightly ahead of the male ones (BEECHEY and SEARLE 1988). In one case, equal rates of nondisjunction were detected in males and females of an intercross involving chromosome 15 Robertsonians (BEECHEY and SEARLE 1988). Others have reported a slightly higher incidence of nondisjunction in male compared with female mice when karyotyping spermatocytes and oocytes (TEASE and FISHER 1991); in contrast however, others have found the reverse (GROPP and WINKING 1981).

The results of the maximum likelihood estimate strongly supported the suggestion that there was a bias against aneuploid gametes in general, especially those that lack an entire chromosome. The best estimate for the frequency of disomic gametes was 10% and for nullisomic gametes 4%. As this estimate was based on the assumption that we would not see any monosomic embryos, as monosomy is usually a more severe phenotype than other aneuploidies (BEECHEY and SEARLE 1988), the frequency of nullisomic gametes was estimated from the incidence of UPD. Previously, this category of nondisjunction would not have been detected because aneuploid embryos have been analyzed by chromosome karyotyping and thus parental origin could not be ascertained. In this study, if UPD were not the result of a conception between a nullisomic and disomic gamete, but instead were the result of mitotic nondisjunction in a trisomic conceptus, then the estimate of 4% for nullisomic gametes would be an overestimate, which only further strengthens the argument for severe selection against nullisomic gametes.

Development of uniparental disomic embryos: Gynogenetic and androgenetic embryos generated from nuclear transplantation experiments (MCGRATH and SOLTER 1983, 1984) can progress to 10.5 dpc(SURANI *et al.* 1986). However, these were exceptional occurrences and most embryos of this type do not survive this long. In these studies, developmental syndromes affecting largely the trophoblast and the embryo respectively, generally arrest normal development at a much earlier stage. Because these embryos have a correct chromosomal complement, the lethality is due to imprinting, namely unequal expression of certain genes on the parental alleles. Studies of intercrosses of mice carrying one Robertsonian chromosome or reciprocal translocation chromosome led to the definition of particular chromosomal regions that show imprinting effects (SEARLE and BEECHEY 1985). Chromosomal regions that showed noncomplementation, *i.e.*, two copies from a single parent could not substitute for a missing copy from the other parent included portions of chromosomes *2, 6, 7, 8, 11, 12* and *17.* Lack of complementation is occasionally nonreciprocal. For example, mice with uniparental disomy (UPD) of mouse chromosome *6* are normal and survive when both chromosomes are of paternal origin but maternal UPD embryos are not viable (SEARLE and BEECHEY 1985; CATTANACH 1986).

For chromosome *7*, complementation studies with a translocation chromosome called *T9H* has established that embryos with paternal duplication, maternal deficiency of distal mouse chromosome *7* die before day 11 post coitum (SEARLE and BEECHEY 1990). Embryos with maternal duplication and paternal deficiency of distal chromosome *7* are growth retarded and die around day 16 post coitum (FERGUSON-SMITH *et al.* 1991). Embryos with maternal duplication of the central portion of chromosome *7* are subject to postnatal lethality whereas paternal duplication has not been associated with an affect (CATTANACH *et al.* 1992). Maternal duplication of the proximal part of chromosome *7* causes neonatal lethality and the corresponding paternal duplication has affects on postnatal growth and viability (SEARLE and BEECHEY 1990; CATTANACH *et al.* 1992). This chromosome must therefore be a very rich source of imprinted genes. It is not clear at what developmental stage the whole chromosome *7* maternal or paternal UPD is lethal. In these molecular studies, the latest stage at which normally developed maternal or paternal UPDs of chromosome *7* were observed was at 8 dpc. Our results are therefore consistent with the wealth of previous data demonstrating that chromosome *7* is imprinted and that particular genes on that chromosome are expressed exclusively from one parental allele or the other.

For chromosome *18* UPD embryos, the rate of retardation was 46%, higher than in the normal genotype category (18%). This was an unexpected result because chromosome *18* is not known to have imprinted genes. In one cross involving the *T50H* and the *T18H* translocation chromosomes, most of the maternal and the most distal portions of the paternal chromosome *18* were shown not to exhibit imprinting effects (BEECHEY *et al.* 1991). This left a small part of the maternal chromosome and most of the paternal chromosome untested for imprinting. Thus, one explanation for these observations is that the untested portions of chromosome *18* do indeed have one or more imprinted genes.

CATTANACH and BEECHEY have recently argued that in crosses involving translocation chromosomes, differences in the frequency of paternal *vs.* maternal UPD offspring may not be the result of imprinting at all but instead may reflect biases against aneuploid gametes during maternal or paternal gametogenesis (CATTANACH and BEECHEY 1994). Based on evidence from other translocation chromosomes, the discordance between maternal *vs.* paternal frequencies was attributed to either nonbiological causes or to nonrandom segregation of chromosomes to the polar bodies in the oocytes of the female (EICHENLAUB-RITTER and WINKING 1990; TEASE and FISHER 1991; CATTENACH and BEECHEY 1994). In our cross, indeed there were only two paternal chromosome *18* UPD embryos out of 429 compared with 12 maternal UPDs, suggesting an increased recovery of UPD embryos of maternal *vs.* paternal origin. An excess of maternal over paternal UPD among offspring has also been seen in studies of chromosome *1*, while a reciprocal excess of paternal over maternal has been seen with chromosomes *5*, *9* and *14* (CATTANACH and BEECHEY 1994). However, the major argument for imprinting of chromosome *18* rather than nonrandom segregation during gametogenesis is the observation that nearly half of all chromosome *18* UPD embryos showed growth retardation, regardless of whether maternal or paternal UPD for chromosome *18* was present. Thus, although there appears to be an imbalance in the frequency of paternal versus maternal UPD for chromosome *18* in our cross, which may reflect in part some nonrandom segregation of chromosomes to polar bodies and oocytes, the occurrence of growth retardation in UPD embryos of either parental origin serves as evidence for an imprinting effect for this chromosome. A number of other translocation chromosomes exist that could be used to test particular regions of chromosome *18* more thoroughly for imprinted genes.

Clustering of nondisjunction events: The distribution of embryos with nondisjunction events (Table 3) suggests that they are clustered among certain matings and therefore not distributed randomly among the 76 litters. Statistical analyses were performed on these data to asses the validity of this observation. In the sample of 429 genotyped embryos, the 65 trisomies, 25 UPDs and seven double trisomies gave a chi-squared statistic of 79.81389 and a P value of 0.009. The generalized likelihood ratio P value for this data set was 0.013, similar to that of the chi-squared statistic. This suggests that the nondisjunction events in these mice were not distributed randomly among the litters, but that some litters were more likely to contain aneuploid or UPD embryos than others. The factors that could have caused this clustering are not clear. Because females in this cross were sacrificed at the time embryos were harvested, we could not determine if females in which we observed clustering of nondisjunction events were more likely to have more clustering of nondisjunction events in future litters. The distribution of nondisjunction events was not equal between all of the males. Forty-nine out of the 76 litters had a father with an assigned number (Table 5). Among the 12 different males, most produced approximately equal numbers of litters with entirely normal embryos and litters with embryos with one or more nondisjunction event. However, two outstanding males produced six and eight litters containing one or more offspring with nondisjunction events (Table 5). It is therefore possible that events in male meiosis and gametogenesis as well as in female meiosis, gametogenesis, or pregnancy, may contribute to the nonrandom distribution of nondisjunction events seen in these litters.

There are a number of possible explanations for the clustering. These explanations include mitotic nondisjunction leading to germline mosaicism for aneuploid gamete precursors in some individuals, increased tendency to meiotic nondisjunction in some individuals, or factors that allow some females to carry chromosomally abnormal fe-

TABLE 5

Distribution of litters among 12 F_1 males

Male	Litter with nondisjunction	Litter without nondisjunction
CXB #9*	8	1
CXB #1	2	3
RXD #3	1	1
CXB #8	3	2
RXD #1	1	0
BX3 #1	2	4
BX3 #3*	6	2
DXR #2	2	0
DXR #7	1	1
BX3 #4	4	1
RXD #2	3	0
RXD #4	1	0
Total	34	15

The genotypes of males and the number of litters with one or more and without nondisjunction events are detailed. Of the 76 litters, 49 litters have fathers of an assigned number, for the remaining 27, the strain of the father only is recorded and these data do not appear here. BXC males were sterile and 3XB males were not used.

tuses longer than other females. As we did not observe clustering of aneuploid or UPD embryos in crosses involving one or the other F_1 hybrid strain of mice in our cross (Table 1), we could draw no conclusions as to the role, if any, played by genetic background in the frequency of nondisjunction or in generating any differences in a putative tolerance to aneuploidy. Consequently, the mechanism of the observed clustering remains obscure.

In humans, there also appears to be clustering of nondisjunction events in sibships in which trisomy *21* has occurred. The recurrence risk for nontranslocation trisomy *21* in the offspring of young women is elevated three- to fivefold over the general population risk for age-matched control women (Gardner and Sutherland 1989). Although one has to be cautious in comparing the clustering of aneuploid offspring in humans with that in the mouse, the notion that there could be some common mechanism that explains either a predisposition to nondisjunction and/or aneuploid embryo survival is suggested by these observations.

In summary, the use of Robertsonian translocation chromosome harboring mice can be used to study elevated rates of nondisjunction in meiosis. The frequency of nondisjunction in the gametes was inferred from an examination of the genotypes of the embryos. These kinds of studies are useful for examining the frequency of, and potentially the mechanisms involved in, gamete selection and success in mice. In addition, the maternal and paternal UPD embryos generated in this cross will be used to search for additional imprinted genes on chromosome *7* and to explore chromosome *18* for imprinted loci as well.

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