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The maternal DDK syndrome phenotype is determined by modifier genes that are not linked to *Om*

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Abstract. The DDK syndrome is a polar, early embryonic lethal phenotype caused by incompatibility between a maternal factor of DDK origin and a paternal gene of non-DDK origin. Both maternal factor and paternal gene have been mapped to the *Om* locus on mouse Chromosome (Chr) 11. The paternal contribution to the syndrome has been shown to segregate as a single locus. Although the inheritance of the maternal contribution has not been characterized in depth, it as been assumed to segregate as a single locus. We have now characterized the segregation of the DDK fertility phenotype in over 240 females. Our results demonstrate that females require at least one DDK allele at *Om* to manifest the syndrome. However, the DDK syndrome inter-strain cross-fertility phenotype of heterozygous females is highly variable and spans the gamut from completely infertile to completely fertile. Our results indicate that this phenotypic variability has a genetic basis and that the modifiers of the DDK syndrome segregate independently of *Om*.

Introduction

The DDK syndrome (Babinet et al. 1990) is a polar early embryonic lethal phenotype first observed when females from the DDK inbred strain were mated to males of many other inbred strain (Tomita 1960; Wakasugi et al. 1967; Wakasugi 1973, 1974). The embryos begin to die around the morula to blastocyst stage because of an incompatibility between a cytoplasmic factor of DDK maternal origin and a paternal non-DDK ("alien") gene (Babinet et al. 1990; Mann 1986; Renard and Babinet 1986). Both maternal and paternal genes have been mapped to the Ovum mutant (*Om*) locus on mouse Chr 11 (Baldacci et al. 1992, 1996; Cohen-Tannoudji et al. 1996; Pardo-Manuel de Villena et al. 1997; Sapienza et al. 1992). Although the molecular identity of the maternal and paternal components is unknown, RNA microinjection experiments indicate that the DDK maternal factor is present as an RNA in DDK oocyte cytoplasm (Renard et al. 1994). Biochemical and cellular studies have also demonstrated the presence of reduced gap junctional communication and low intracellular pH in DDK eggs fertilized by alien sperm and suggested a relationship with the early embryonic death (Buehr et al. 1987; Leclerc et al. 1994).

The fraction of embryos that dies in the DDK syndrome depends on the "alien" strain partner used in inter-strain mating (Wakasugi et al. 1967; Wakasugi 1974). Two parameters have been used to characterize the fertility phenotype of crosses involving the DDK strain: i) determination of the fraction of viable embryos found early in development by use of in vivo and in vitro methods, and ii) litter size at birth. Crosses involving the C57BL/6 (B6) and DDK inbred strains and their reciprocal F_1 hybrids have been characterized by both approaches (Sapienza et al. 1992; Pardo-Manuel de Villena et al. 1996, 1997; Wakasugi 1973, 1974). These crosses have been classified into four categories based on the extent of the lethality. Crosses between DDK females and B6 males have been considered as "lethal" because up to 95% of the embryos die (it should be noted that the rare survivors of these crosses are both viable and fertile). Crosses between F_1 females and B6 males and between DDK females and F_1 males result in the death of approximately 50% of the embryos and have been classified as "semilethal". Crosses between B6 females and DDK males, F_1 females and DDK males, and between B6 females and F_1 males are viable. Lastly, intercrosses between F_1 males and females result in the death of 25% of the embryos.

The paternal component of the DDK syndrome segregates as a single locus, as demonstrated by the analysis of a large number of backcross males segregating for *Om* (Baldacci et al. 1992, 1996). These studies define a 0.28-cM (0.0 – 1.6 cM, 95% confidence interval) candidate interval for the paternal gene (*Om*), located between *Scya2* and *D11Mit35/36* (Baldacci et al. 1996). The single-locus nature of the paternal contribution to the syndrome is supported by the strong correlation between offspring survival and the paternal *Om* allele inherited by embryos in semilethal backcrosses between DDK females and F_1 males (Sapienza et al. 1992; Wakasugi 1974). In such crosses the vast majority of survivors inherit the paternal DDK allele at *Om* (Om^k), while the embryos that die are inferred to have inherited the B6 allele at *Om* (Om^b).

The inheritance of the maternal contribution is less well characterized. Although backcrosses between F_1 females and B6 males have been studied by us and others, the semilethal nature of these crosses is difficult to explain. In brief, if F_1 females synthesize the maternal DDK factor before fertilization, as do DDK females, the fact that 50% of the embryos survive requires either unusual regulation of *Om* transcription in ova from F_1 females or epistasis. Moreover, in contrast to the 1:1 Mendelian ratio of inheritance of alleles at *Om* predicted by Wakasugi (1974) and the segregation distortion in favor of the Om^b allele predicted by Sapienza and coworkers (1992), we found that surviving offspring from the semilethal backcross between reciprocal F_1 hybrid females and B6 males show a modest but highly significant and reproducible segregation distortion in favor of the maternal Om^k allele (Pardo-Manuel de Villena et al. 1996, 1997). We have proposed previously that the uncoupling of lethality and *Om* genotype among the offspring of semilethal F_1 backcross females must reflect an unusual expression pattern of the maternal gene or an unusual transmission or sequestration of the DDK maternal "factor" within individual ova (Pardo-Manuel de Villena et al. 1996, 1997). It should also be noted that only a small number of N_2 females have

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been characterized for their DDK syndrome phenotype (Pardo-Manuel de Villena et al. 1997; Wakasugi 1974) and that the genotype at *Om* was unknown in one of these experiments (that of Wakasugi 1974).

To further characterize the maternal contribution to the DDK syndrome, we decided to test formally whether the maternal contribution is inherited, as predicted, as a single locus. For this purpose we have defined criteria for the characterization of the fertility phenotype of individual females. This was achieved by generating a large number of observations in control crosses and including a new parameter, "delivery ratio", as a component of the phenotype. Our results demonstrate that, in contrast with prior expectation, the DDK syndrome fertility phenotype of Om^b/Om^k heterozygous females does not segregate as a single locus. Among these females, the DDK syndrome fertility phenotype is the result of an interaction between *Om* and unlinked modifier genes.

Materials and methods

Mouse crosses. The C57BL/6 strain used in the majority of F_1 backcross experiments that have been described previously (Sapienza et al. 1992; Pardo-Manuel de Villena et al. 1996) was obtained from The Jackson Laboratory (Bar Harbor, Me). The C57BL/6 strain used in the present F_1 backcross experiments was obtained from Harlan Sprague Dawley (Indianapolis, IN). Some of the DDK animals used in this study were kindly provided by Charles Babinet from the Institut Pasteur (Paris). In all crosses described in the text, the dam is listed first and the sire is listed second. The F_1 × DDK and F_1 × B6 backcrosses and 24 of the (F_1 × B6) N_2 females have been described previously (Pardo-Manuel de Villena et al. 1996, 1997; Sapienza et al. 1992). Experimental females used in this study were obtained by backcrossing and intercrossing (C57BL/6 × DDK)F_1 and (DDK × C57BL/6)F_1 individuals and their descendants. All mice described in this experiment were treated according to the recommendations of the Canadian Council on Animal Care, or the IUCAC of Temple University School of Medicine.

Microsatellite markers and genotype determination. DNA extraction from tail biopsies, gel electrophoresis, and autoradiography were all performed as described previously (Maniatis et al. 1982; Hogan et al. 1986). Oligonucleotide primers for the *D11Mit36* (Dietrich et al. 1994) locus were obtained from Research Genetics (Hunstville, Ala.), and genotypes were determined as suggested by the manufacturer. Alleles at the *Scya2* locus were identified as described previously (Aitman et al. 1991).

Phenotype determination. The following breeding protocol was used to determine the fertility phenotype: females were exposed to an inbred male (either B6 or DDK) for at least a 3-week period (average 10 weeks), and the cages were checked once a day for the presence of newborn pups. Females that failed to give litters with the first male were subsequently placed with another male of the same inbred strain. Two parameters were recorded for each mating: litter size and delivery ratio. Litter size was determined by counting pups at birth, regardless of their status (alive or dead). We calculated the average litter size for each female by dividing the total number of offspring by the number of litters produced in mating to each type of inbred male. When females were classified by their genotype at *Om* and the type of inbred male to which they were mated, the litter size was used to estimate two parameters: the cross average litter size, i.e., total number of offspring divided by total number of litters, and the female average litter size, which is the sum of the average litter size of each female divided by the number of females. The delivery ratio was estimated by dividing the number of days that a female was pregnant (assumed to be 21 days for each pregnancy) by the total number of days that the female was exposed to males of the same inbred strain; the pregnancy period was included when the male was removed from the cage prior to delivery. Females were bred only until they reached 10 months of age to avoid any reduction of fertility associated with aging. The delivery ratio attempts to account for the proportion of litters of size zero, because average litter size is based only on litters of size greater than or equal to one.

Three control crosses, F_1 × DDK, F_1 × B6, DDK × B6, were established to determine the fertility phenotype in viable, semilethal, and lethal crosses, respectively. In addition to the females that were characterized as described above, we incorporated the data from 18 F_1 females mated to DDK males, 72 F_1 females mated to B6 males, and 23 DDK females mated to B6 males, which have been published previously and for which only the litter sizes were available (Pardo-Manuel de Villena et al. 1996, 1997). The distributions of the average litter size of all control females are shown at the bottom of Fig. 1.

In addition to the average litter size and delivery ratio of each female, we used the occurrence of a litter of more than seven pups as an exclusion criterion for semilethal and lethal crosses. Litters of more than seven pups were rare events in semilethal (F_1 × B6) control crosses [5 litters out of 307 (1.6%) and 5 females out 113 (4.4%)] (Pardo-Manuel de Villena et al. 1997), and were not observed in lethal (DDK × B6) control crosses in the present experiment (0/32 litters and 0/45 females). As shown in Fig. 1, when individual females were categorized according to their average litter size, delivery ratio, and the occurrence of litters of more than seven pups (symbols within squares), females in lethal, semilethal, and viable crosses cluster in three minimally overlapping groups. We used the distribution pattern of these clusters and the female average litter size distribution (Fig. 1, bottom) to establish a set of criteria that define five mutually exclusive phenotypic categories:

Fertility phenotype	Average litter size	Occurrences (litter > 7)	Delivery ratio
1. Lethal	× < 2.5	No	< 0.5
2. Lethal/F_1	× < 2.5	No	> 0.5
	2.5 < × < 6.5	No	< 0.5
3. F_1	2.5 < × < 6.5	No	> 0.5
4. F_1/Viable	6.5 < ×	Yes	< 0.5
	× < 6.5	Yes	> 0.5 or < 0.5
5. Viable	6.5 < ×	Yes/No	> 0.5

This phenotype classification is comprised of ordered categories from less fertile to more fertile. The criteria for three of these phenotypic categories, lethal, F_1 and viable, were designed to group the majority of females in lethal, semilethal, and viable control crosses into modal categories (73%, 81%, and 73% of control crosses, respectively). The other two categories represent intermediate phenotypes between the three modal categories. Our criteria were also designed to minimize the number of phenotypically discordant females in control crosses, i.e., those that fall in a phenotypic category that is different from either the mode or any of the intermediate categories contiguous to the modal for each control cross. As shown in Table 1, approximately 5% of females from the control crosses have a discordant phenotype (5%, 2%, and 13% in lethal, semilethal, and viable crosses, respectively).

Statistical analysis. As shown in Fig. 1, the average litter size and delivery ratio distributions of some of the control crosses are strongly skewed. Therefore, instead of comparing only the means of the distributions to determine whether experimental and control crosses are significantly different, we also used the Mann-Whitney test (Mann and Whitney 1947). The two-tailed test was performed as described by Conover (1971). The null hypothesis was that experimental crosses should not be significantly different from the appropriate control cross at the 0.05 level of significance. Fisher's Exact Probability Test was performed with the S-PLUS software (MathSoft 1996).

Results

We determined the genotype of 244 experimental females by PCR amplification of alleles at *Scya2* and *D11Mit36.* These two markers flank the 0.28-cM candidate interval for *Om* (Baldacci et al. 1996). Females that did not carry recombinant chromosomes were assigned directly the corresponding *Om* genotype: Om^b/Om^b, Om^b/Om^k, or Om^k/Om^k. Females carrying recombinant chromosomes within the region were typed for additional markers and *Om* genotype was inferred from our refined map of this region (F. Pardo-Manuel de Villena and C. Sapienza, unpublished). Females of each *Om* genotype were mated to B6, DDK, or both types of males, generating six experimental crosses. The expectation for the fertility phenotype of each cross was based on a single locus model for the DDK syndrome and was determined on the basis of the *Om*

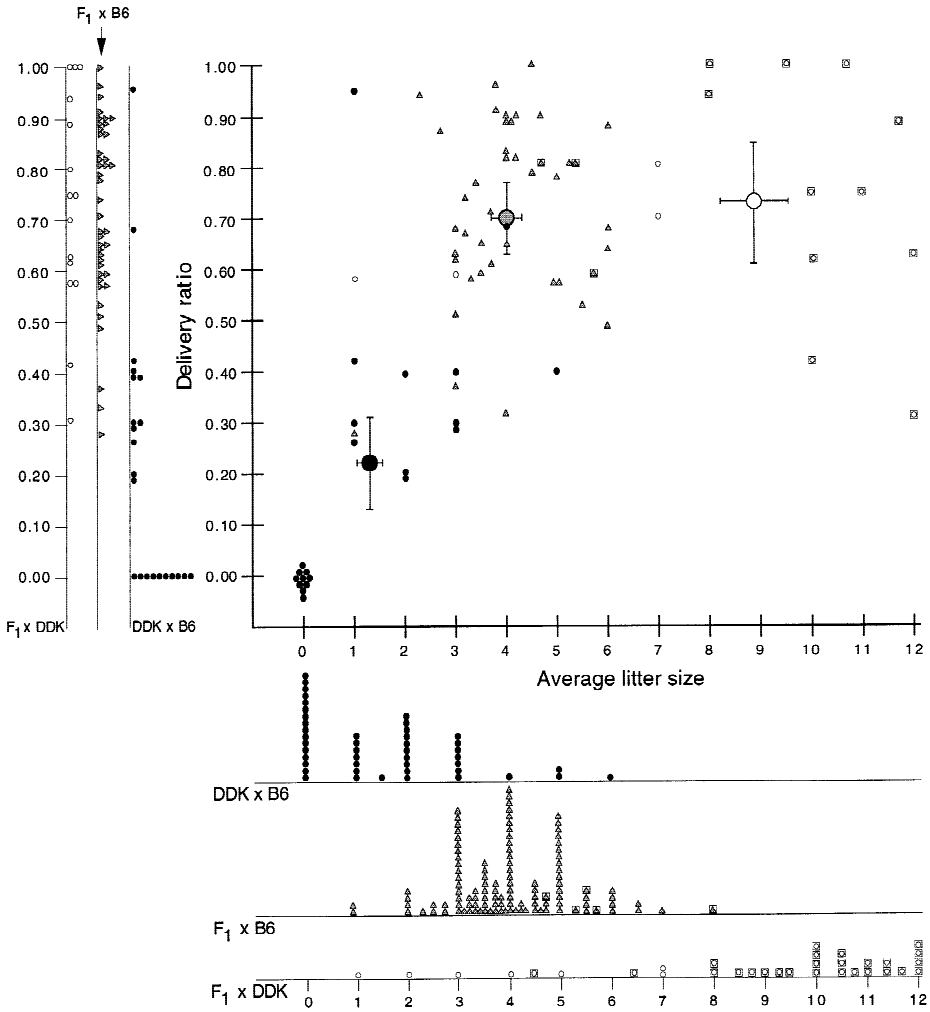

Fig. 1. Average litter size and delivery ratio in control crosses. The horizontal axis indicates average litter size, while the vertical axis represents delivery ratio. Each circle or triangle represents an individual female. Black circles represent females in DDK × B6 lethal crosses; triangles represent females in F_1 × B6 semilethal crosses; and open circles represent females in F_1 × DDK viable crosses. A circle or a triangle within a square indicates a female with at least one litter of more than seven pups. The bottom of the figure depicts the average litter size distribution of 45 DDK females mated to B6 males, 114 F_1 females mated to B6 males, and 31 F_1 females mated to DDK males. The delivery ratio parameter was available only for 22, 42, and 15 of these females, respectively, and therefore only those females are represented in the scatter plot. Large circles with standard error bars represent the averages of lethal (filled circles), semilethal (shaded circles), and viable (open circles) control crosses.

Table 1. The DDK syndrome fertility phenotype. The table shows number of litters, number of offspring, cross average litter size, number of females, female average litter size, average delivery ratio (averages are given ± standard errors), and observed fertility phenotype distribution for the three control crosses and the six experimental crosses characterized in this study. The expected fertility phenotype of each cross is based on the genotypes of both dam and sire at *Om.* The observed fertility phenotype distribution indicates the number of females that are categorized into each of the five phenotypic classes as described in the Material and methods.

Cross	Expected fertility phenotype	No. litters	No. offspring	Cross average litter size	No. females	Female average litter size	Average delivery ratio	Fertility phenotype distribution				
								Lethal	Lethal/F_1	F_1	F_1/Viable	Viable
1. F_1 × DDK	Viable[a]	66	594	9.0 ± 0.4	15[b]	8.7 ± 0.8	0.73 ± 0.11	—	1	1	2	11
2. F_1 × B6	F_1[a]	307	1214	4.0 ± 0.1	42[b]	4.1 ± 0.3	0.72 ± 0.07	1	4	34	4	—
3. DDK × B6	Lethal[a]	32	75	2.3 ± 0.3	22[b]	1.3 ± 0.2	0.22 ± 0.09	16	5	1	—	—
4. Om^b/Om^b × DDK	Viable	21	183	8.7 ± 0.6	11	9.4 ± 0.9	0.73 ± 0.13	—	—	—	2	9
5. Om^b/Om^b × B6	Viable	101	855	8.5 ± 0.3	40	8.6 ± 0.5	0.70 ± 0.07	—	—	4	9	27
6. Om^b/Om^k × DDK	Viable	88	740	8.4 ± 0.3	57	8.4 ± 0.4	0.78 ± 0.05	—	—	9	5	43
7. Om^b/Om^k × B6	F_1	181	628	3.4 ± 0.1	101	2.9 ± 0.2	0.51 ± 0.05	33	25	38	1	4
8. Om^k/Om^k × DDK	Viable	122	1014	8.3 ± 0.3	56	8.5 ± 0.4	0.78 ± 0.06	—	1	5	5	45
9. Om^k/Om^k × B6	Lethal	44	106	2.4 ± 0.2	40	1.3 ± 0.2	0.28 ± 0.07	24	15	1	—	—

[a] The fertility phenotypes in these crosses are defined as viable, semilethal, and lethal in the original description of the DDK syndrome (Pardo-Manuel de Villena et al. 1997; Sapienza et al. 1992; Wakasugi 1973, 1974) and are used as controls in this experiment.

[b] In addition to the females listed in each cross, we incorporated the data for the number of litters, the number of offspring, and the cross average litter size from 18 F_1 females mated to DDK males, 72 F_1 females mated to B6 males, and 23 DDK females mated to B6 males that have been published previously and for which only the litter sizes were available (Pardo-Manuel de Villena et al. 1996, 1997).

genotypes of the dam and sire (Table 1). Note that crosses between experimental females and B6 males are the only crosses that provide information about the DDK syndrome fertility phenotype. Mating experimental females to DDK males serves as an internal control to detect differences in fertility that are not related to the DDK syndrome.

Although the DDK maternal factor is linked to Om, the fertility phenotype of heterozygous females is variable. The results shown in Table 1 confirm that the maternal DDK factor is linked to the *Om* locus. Only females with at least one Om^k allele show a reduction in fertility when mated to B6 males in comparison with DDK males (compare cross 6 with 7, and 8 with 9; Table 1). In

Table 2. The variability in the fertility phenotype of Om^b/Om^k females is related to the DDK syndrome. Only Om^b/Om^k females that have been mated to both B6 and DDK inbred males are included in this table. The females have been classified in five phenotypic groups on the basis of their observed fertility phenotype when mated to B6 males (see observed fertility phenotype column and cross 7 in Table 1). The expected fertility phenotype of each cross is based on the genotypes of both dam and sire at *Om*. The observed fertility phenotype distribution indicates the number of females that are categorized into each of the five phenotypic classes as described in the Material and methods.

		Om^b/Om^k × B6				Om^b/Om^k × DDK							
									Fertility phenotype distribution				
Cross	No. females	Expected fertility phenotype	Observed fertility phenotype	Female average litter size	Average delivery ratio	Expected fertility phenotype	Female average litter size	Average delivery ratio	Lethal	Lethal/F_1	F_1	F_1/Viable	Viable
10.	14	F_1	Lethal	0.8 ± 0.2	0.17 ± 0.10	Viable	8.2 ± 0.8	0.75 ± 0.12	–	–	3	2	9
11.	13	F_1	Lethal/F_1	2.7 ± 0.5	0.55 ± 0.14	Viable	7.8 ± 0.7	0.86 ± 0.10	–	–	3	1	9
12.	18	F_1	F_1	3.9 ± 0.5	0.72 ± 0.11	Viable	8.7 ± 0.7	0.79 ± 0.10	–	–	3	2	13
13.	1	F_1	F_1/Viable	5.0 ± 2.2	0.90 ± 0.30	Viable	10.0 ± 3.2	0.95 ± 0.21	–	–	–	–	1
14.	3	F_1	Viable	7.8 ± 1.6	0.65 ± 0.28	Viable	10.0 ± 1.8	0.73 ± 0.26	–	–	–	–	3

contrast, the fertility of Om^b/Om^b females is independent of the males to which they are mated (compare cross 4 with 5, Table 1). The results in Table 1 indicate that the expectations for the four viable experimental crosses (crosses 4, 5, 6, and 8; Table 1) and the lethal experimental cross (cross 9, Table 1) are fulfilled. In these five crosses the litter sizes, the delivery ratios, and the fertility phenotype distributions do not differ from the values of the corresponding control crosses. However, the cross between Om^b/Om^k females and B6 males (cross 7, Table 1), in which females are expected to behave similarly to the F_1 females in the F_1 × B6 semilethal control cross (cross 2, Table 1), shows reductions in average litter size and delivery ratio. In addition, the fertility phenotype distribution of females in this cross is significantly different from the corresponding control ($P << 0.0001$). Inspection of the fertility phenotype distribution shows that there is a strong reduction (79% expected, 38% observed) in the expected modal phenotype, "F_1", while there are marked increases in the proportion of females with the lethal phenotype (2% expected, 33% observed), and females with the lethal/F_1 intermediate phenotype (9% expected, 25% observed). Thus, there are many more Om^b/Om^k heterozygous females who have poor breeding performance when crossed with B6 males, than expected (11% expected, 57% observed).

The variability in the fertility phenotype of heterozygous females is related to the DDK syndrome. To determine to what extent this result is related to the DDK syndrome rather than low fertility of individual females per se, we subdivided the 49 Om^b/Om^k females that had been mated to both B6 and DDK males according to the fertility phenotype (lethal, lethal/F_1, F_1, F_1/viable, and viable) observed when these females were mated to B6 males. As shown in Table 2, the average litter size and the delivery ratio of crosses between females from each of these five phenotypic categories and DDK males (cross 10, 11, 12, 13, and 14; right side of Table 2) do not differ from the control viable cross (cross 1, Table 1). Moreover, there is no significant difference between the fertility phenotype distributions of these five groups when mated to DDK males (crosses 10, 11, 12, 13, and 14; Table 2) (Fisher exact test, $P = 1$). Therefore, there is no correlation between the fertility phenotype of these females mated to B6 males and their fertility phenotype when mated to DDK males. We conclude that the discordant fertility phenotype among Om^b/Om^k females is related to the DDK syndrome and not simply lower fertility overall.

Genetic background determines the fertility phenotype of heterozygous females. Because the inbred strain mating partner used to generate the experimental Om^b/Om^k females was B6 in some cases and DDK in others, this variable can be used to test whether the genetic background of an individual female is related to her fertility phenotype when mated to B6 males. We classified the Om^b/Om^k females into three groups: i) females whose dam or sire was from the B6 inbred strain (cross 15, Table 3), ii) females whose dam or sire was from the DDK inbred strain (cross 16, Table 3), and iii) females whose dam and sire were of mixed B6/DDK background (cross 17, Table 3). Females from the first group could not be homozygous for DDK alleles at any locus and, on average, should have a higher frequency of B6 alleles in their background. Females from the second group could not be homozygous for B6 alleles at any locus and should have a higher DDK genetic background content. As shown in Table 3, 55 out 101 Om^b/Om^k females were obtained from backcrosses to the B6 inbred strain, 24 were obtained from backcrosses to the DDK inbred strain, and 22 by intercrossing. Heterozygous Om^b/Om^k females obtained from backcrosses to B6 (cross 15, Table 3) have lower average litter size and delivery ratio than females obtained from backcrosses to DDK (cross 16, Table 3). Inspection of the fertility phenotype distributions on Table 3 indicates that females obtained from backcrosses to B6 (cross 15, Table 3) have poorer breeding performance than females obtained from backcrosses to DDK (cross 16, Table 3).

As a further test of the influence of the genetic background on the fertility phenotype, we compared two different sets of Om^b/Om^k heterozygous N_2 females. The first set is composed of 24 N_2 females obtained by backcrossing F_1 females to B6 males (cross 18, Table 4). The second set is composed of 14 females obtained by backcrossing F_1 females to DDK males (cross 19, Table 4). As shown in Table 4, the observed fertility phenotype distributions of both cross 18 and cross 19 are significantly different (Fisher exact test, $P < 0.033$).

Discussion

The early developmental death of embryos with incompatible ova-cytoplasm and "alien" paternal *Om* allele is the defining characteristic of the DDK syndrome. Two approaches have been used to characterize the extent of this lethality: i) viability of embryos in early development by in vivo and in vitro methods, and ii) litter size at birth. Previously we have used the latter approach to map the paternal gene (Sapienza et al. 1992), to confirm directly the linkage between maternal and paternal genes (Pardo-Manuel de Villena et al. 1997), and to characterize the inheritance of alleles at *Om* through F_1 females (Pardo-Manuel de Villena et al. 1996, 1997). Until now, we have relied on the average litter size at birth and the presence of litters of more than seven pups to determine the female fertility phenotype (Pardo-Manuel de Villena et al. 1997). This approach was useful when only two phenotypes, F_1 and viable, were expected. However, in the present study viable, semilethal (F_1), and lethal phenotypes are all present. The characterization of the lethal phenotype presents additional problems because: i) 36% of the DDK females (16 out of 45) fail to have any litters when mated to B6 males, and ii) the average litter size distribution of DDK females that do have litters when mated to B6 males overlaps extensively with the average litter size distribution of the F_1 × B6 semilethal control backcross (Fig. 1).

To account for females with an average litter size of zero and to distinguish between lethal and semilethal crosses, we have in-

Table 3. The fertility phenotype of Om^b/Om^k females depends on their genetic background. Fertility phenotype of 101 Om^b/Om^k females that have been mated to B6 males and classified according to whether they derive in the last generation from a backcross to a B6 inbred parent, a DDK inbred parent, or both parents of mixed B6/DDK background. The expected cross phenotype is based on the genotypes of both parents at *Om*.

	Parental inbred strain	Expected fertility phenotype	No. litters	No. offspring	Cross average litter size	No. females	Female average litter size	Average delivery ratio	Fertility phenotype distribution				
									Lethal	Lethal/F_1	F_1	F_1/Viable	Viable
15. Om^b/Om^k × B6	B6	F_1	89	280	3.1 ± 0.2	55	2.5 ± 0.2	0.41 ± 0.07	25	12	18	–	–
16. Om^b/Om^k × B6	DDK	F_1	43	177	4.1 ± 0.3	24	4.0 ± 0.4	0.73 ± 0.09	–	6	14	1	3
17. Om^b/Om^k × B6	mixed	F_1	48	164	3.4 ± 0.3	22	2.9 ± 0.4	0.50 ± 0.11	8	7	6	–	1

Table 4. Segregation of the DDK syndrome fertility phenotype among reciprocal N_2 females.

Cross	No. females	Fertility phenotype distribution				
		Lethal	Lethal/F_1	F_1	F_1/Viable	Viable
18. (Om^b/Om^k)(F_1 × B6) N_2 × B6	24	6	5	13	–	–
19. (Om^b/Om^k)(F_1 × DDK) N_2 × B6	14	–	3	8	–	3

troduced an additional parameter in the phenotype determination, the delivery ratio. This parameter estimates the time required for a female to successfully give birth to a litter, independent of the litter size. Delivery ratio, as defined in this study, is related to the "productive mating" parameter (Silver 1995) used to determine the reproductive performance of inbred strains and to the "pregnancy rate" parameter described by Wakasugi (1973). Table 1 indicates that the delivery ratio in the lethal control cross (cross 3, Table 1 and Fig. 1) is significantly smaller than in viable and semilethal crosses (cross 1 and 2, Table 1 and Fig. 1; two-tailed Mann-Whitney test, $p < 0.0002$). Moreover, only two of 22 DDK females mated to B6 males had a delivery ratio larger than 0.5 (Fig. 1), while the vast majority of F_1 females in both semilethal and viable crosses have delivery ratios larger than 0.5 (90% and 85% of females from semilethal and viable crosses, respectively). The combined use of average litter size, occurrence of a litter with more than seven pups, and delivery ratio has allowed us to establish a set of criteria that are exclusive and representative of each of the three control female fertility phenotypes, lethal, semilethal (F_1) and viable (see Fig. 1 and Materials and methods).

To date, all genetic models of the DDK syndrome are based on the premise that only a single locus, *Om*, is relevant for the fate of the embryos. The single locus nature of the paternal contribution to the syndrome is well documented (Baldacci et al. 1992, 1996; Sapienza et al. 1992; Wakasugi 1974), but the single-locus inheritance of the maternal contribution had not been tested formally. Under a single-locus model, the female fertility phenotype should be determined only by the genotype at *Om*.

We have analyzed the fertility phenotype of 244 females (47 Om^b/Om^b, 109 Om^b/Om^k, and 88 Om^k/Om^k that have an otherwise heterogeneous mix of B6 and DDK alleles in their genetic background) by mating them to B6 and DDK males. Our results confirm that the maternal DDK factor required for lethality is linked to the paternal gene (Pardo-Manuel de Villena et al. 1997). As expected, Om^b/Om^b females do not show differential fertility when mated to B6 versus DDK males. Moreover, the fertility phenotype of these females when mated to B6 males (cross 5, Table 1) is not different from the viable control cross (cross 1, Table 1). As expected, the fertility phenotype of females with one (Om^b/Om^k) or two (Om^k/Om^k) DDK alleles at *Om* is dependent on the type of male to which they are mated. However, the maternal contribution does not segregate as single locus because the fertility phenotype of Om^b/Om^k heterozygous females is variable. Direct comparison of the fertility phenotype distributions of F_1 × B6 (cross 2, Table 1) and Om^b/Om^k × B6 (cross 7, Table 1) crosses demonstrates that there is a marked increase (2% expected, 37% observed) in the percentage of females with discordant phenotypes (lethal and viable) in the latter cross.

If the changes in the fertility phenotype were independent of the DDK syndrome, one should observe a correlation between the fertility phenotype when females are mated to B6 males and the fertility phenotype when these same females are mated to DDK males. This is not the case, because Om^b/Om^k females have a viable fertility phenotype when mated to DDK males regardless of their fertility phenotype when mated to B6 males (Table 2). The most extreme case is represented by cross 10, in which mating to B6 males results in a 0.8 ± 0.2 female average litter size and a 0.17 ± 0.10 average delivery ratio (both values in the expected range for a lethal cross) while the female average litter size (8.2 ± 0.8) and average delivery ratio (0.75 ± 0.12) in mating to DDK males are characteristic of viable crosses (9.1 ± 0.8 and 0.73 ± 0.12, respectively; see cross 1, Table 1).

We have demonstrated that the increase in the percentage of females with the discordant lethal fertility phenotype is highly significant and has a genetic basis. However, the increase in the percentage of Om^b/Om^k females with the other discordant fertility phenotype, viable, is modest (0% expected, 4% observed; cross 7, Table 1) and could be ascribed to variability in the phenotype determination. However, three observations indicate that this second discordant phenotype is also due to genetic factors. First, the majority of viable females originate from backcrosses to DDK males (Table 3), indicating that there is a relationship between this phenotype and the genetic background. Second, in (Om^b/Om^k) (F_1 × DDK) N_2 females (cross 19, Table 4) there is a marked increase (0% expected, 21% observed) in the percentage of females who have the viable phenotype with respect to the F_1 × B6 control (cross 2, Table 1). This increase provides a major contribution to the significance level of the heterogeneity test between the fertility phenotype distribution of (Om^b/Om^k) (F_1 × DDK) N_2 × B6 cross and the control cross (cross 2, Table 1). Third, the ratio of segregation of discordant phenotypes in reciprocal N_2 females is similar (25% in cross 18 and 21% in cross 19, Table 4), although only one discordant phenotype segregates in each cross (lethal in cross 18 and viable in cross 19). We conclude that the fertility phenotype of Om^b/Om^k females mated to B6 males could be any of the three modal phenotypes, lethal, F_1, or viable, and that the variability has a genetic basis. In other words, the observed fertility phenotype of an Om^b/Om^k heterozygous female is determined by her genotype at a modifier locus or loci.

The segregation of the fertility phenotype in N_2 females also provides preliminary information about the number of loci involved in the phenotypic variability. As shown in Table 4, there is a marked increase in the proportion (2% expected, 24% observed) of N_2 females with discordant phenotypes, lethal and viable. This increase in the proportion of females with discordant phenotypes could be explained either by a single modifier locus linked to *Om*

and located 20 cM either proximal or distal, or by multiple loci. We have tested and rejected ($P << 0.001$) the single-locus model after determining the genotypes of 72 Om^b/Om^k females having the three different modal phenotypes, 32 lethal, 36 F_1, and 4 viable, at *D11Mit206* and *D11Mit168* [data not show; position 19.6 cM and 71 cM respectively (Montgomery et al. 1998)]. We conclude that the fertility phenotype modification in Om^b/Om^k females is a complex process that involves more than one locus. Because the segregation of the phenotype has been characterized in only a modest number of N_2 females (38), an estimate of the number of loci implicated can not be determined with confidence.

Regardless of the number of loci implicated in the phenotype modification, several conclusions may be drawn from our results. First, the modifier genes are able to modify the phenotype of heterozygous females, but not the fertility phenotype of Om^k/Om^k homozygous females. Second, the segregation of fertility phenotype in N_2 females indicates that the discordant lethal and viable phenotypes behave as recessive with respect to the modal F_1 phenotype. Third, B6/B6 homozygosity at some locus in Om^b/Om^k heterozygous females is incompatible with the viable phenotype because none of the 55 females obtained from B6 backcrosses had this phenotype (cross 15, Table 3). Reciprocally, DDK/DDK homozygosity at some locus is incompatible with the lethal phenotype in Om^b/Om^k heterozygous females, because zero out of 24 females obtained in DDK backcrosses had this phenotype (cross 16, Table 3). Our results agree with the observation reported by Baldacci and coworkers (1997) "that heterozygous females at *Om* show a significant distortion in favour of the DDK cytoplasmic factor and that the more the mice have a non-DDK genotype the more they have a DDK phenotype".

We conclude that, although the maternal DDK factor is located at *Om* and at least one Om^k allele is required in order to manifest the DDK syndrome, the maternal contribution to the DDK syndrome does not segregate as a single locus in Om^b/Om^k heterozygous females. The fertility phenotype of Om^b/Om^k heterozygous females results from the interaction between *Om* and unlinked modifier genes.

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