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Female mice of DDK strain are fully fertile in the intersubspecific crosses with *Mus musculus molossinus* and *M. m. castaneus*

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Abstract. The female mice of DDK strain are almost infertile when mated with males from other strains. This phenomenon is caused by the early death of F_1 embryos owing to the incompatibility system attributed to the *ovum mutant* (*Om*) locus on Chromosome (Chr) 11 and known as DDK syndrome. In the present study, DDK females were found to be fully fertile in the intersubspecific matings with the males of two wild mouse-derived strains, MOM (originated from Japanese wild mice, *Mus musculus molossinus*) and Cas (originated from Philippine wild mice, *M. m. castaneus*), indicating that no incompatibility exists between DDK oocytes and spermatozoa of MOM and Cas strains. Furthermore, this compatibility has been confirmed by the following two findings: (1) Normal fertility was shown by the two types of backcrosses, DDK females × F_1 (DDK♀ × MOM♂) males and DDK females × F_1 (DDK♀ × Cas♂) males; and (2) the offspring from these backcrosses segregated equally into the homozygotes and heterozygotes as genotyped by the microsatellite markers closely linked to *Om* locus. MOM and Cas strains would be useful for further investigations on the *Om* locus. On the other hand, the litter size of F_1 [C57BL/6Cr (B6)♀ × Cas♂] females mated with B6 males was about half that of the mating with DDK males. It would be interesting to investigate whether this reduction in fertility is related to the *Om* locus or not.

Introduction

The inbred strain of mice DDK shows a unique property known as "DDK syndrome" (Babinet et al. 1990). The females of the DDK strain are completely or nearly infertile when crossed to males of other strains, while DDK males can normally fertilize the females of other strains. This unidirectional infertility is caused by the death of F_1 embryos owing to failure of blastocyst formation. Based on the genetic analyses, Wakasugi (1974) proposed a hypothesis that the developmental defect of F_1 embryos is caused by an incompatibility system owing to the *ovum mutant* (*Om*) locus. The normal allele at the *Om* locus possessed by the majority of strains produces a cytoplasmic factor O in the oocyte and a sperm factor S, whereas the allele of the DDK strain produces cytoplasmic factor o and sperm factor s. When o and S are present together (o-S), their interaction leads to lethality of the embryos, whereas the embryos having the other three combinations (O-S, O-s and o-s) develop normally. The incompatibility has been experimentally confirmed by transfer of pronuclei and cytoplasm (Mann 1986; Renard and Babinet 1986; Babinet et al. 1990; Cohen-Tannoudji et al. 1996), and the cytoplasmic factor has been found to be present as RNA (Renard et al. 1994). Meanwhile, the incompatibility system due to the *Om* locus was confirmed by other studies, and this locus has been mapped on mouse Chromosome (Chr) 11 (Baldacci et al. 1992, 1996; Sapienza et al. 1992; Cohen-Tannoudji et al. 1996; Pardo-Manuel de Villena et al. 1997). For the convenience of description, the gene symbols Om^d, Om^b, Om^M, and Om^C are used in the present paper to represent the alleles of *Om* locus possessed by DDK, C57BL/6Cr (B6), MOM, and Cas strains, respectively.

It was also hypothesized by Wakasugi (1974) that the cross heterozygous (Om^b/Om^d) females × B6 (Om^b/Om^b) males shows about 50% fertility as compared with the fully fertile cross heterozygous (Om^b/Om^d) females × DDK (Om^d/Om^d) males. However, three recent reports have disclosed the presence of modifier genes modulating the fertility of the heterozygous females mated with alien males. Pardo-Manuel de Villena et al. (1999) suggested the existence of two or more modifiers with reducing or enhancing effects on the basis of the analyses on heterozygous females from two types of backcrosses: F_1 females × B6 males and F_1 females × DDK males. Le Bras et al. (2000) presented genetic evidence indicating that the heterozygous females with background genes of the BALB/c strain produce mostly incompatible lethal embryos when mated with BALB/c males. Zhao et al. (2000) found that embryonic mortality in the heterozygous females mated with B6 males increased stepwise as their genetic background approached that of the B6 strain, confirming the presence of a modifier gene reducing the fertility in the genetic background of B6 strain. From this finding, they have proposed an allelic exclusion hypothesis concerning the synthesis of the cytoplasmic factor of eggs responsible for the DDK syndrome.

It is generally known that female mice of laboratory strains are fertile in the interspecific or intersubspecific crosses, and their crossbreds are used for various genetic studies including gene mapping (Moriwaki et al. 1994). It is interesting to investigate whether the female mice of the DDK strain show normal fertility or reduced fertility in interspecific or intersubspecific crosses. In the present report, we describe (1) fertility of DDK females mated with the males of two wild mouse-derived strains, MOM (established from Japanese wild mice, *M. m. molossinus*) and Cas (established from Philippine wild mice, *M. m. castaneus*); (2) fertility of their F_1 hybrids; and (3) analyses of the inheritance of the microsatellite markers closely linked to the *Om* locus from F_1 hybrids to backcross N_2 offspring.

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Table 1. Reproductive performance in intersubspecific crosses involving five laboratory mouse strains, DDK, C57BL/6Cr (B6), C3H/HeN (C3H), CBA/J (CBA), and DBA/2J (DBA), and two wild mouse-derived strains, MOM and Cas.

Types of crosses[a]			No. of females mated	No. of females with litter(s)	Parturition rate (%)[b]	No. of litters born	Average litter size[c]
Dam		Sire					
DDK	×	MOM	14	12	85.7	21	7.8 ± 0.4[n.s]
B6	×	MOM	11	9	81.8	14	9.8 ± 0.7
C3H	×	MOM	12	11	91.7	16	9.7 ± 0.6
CBA	×	MOM	6	6	100.0	9	7.7 ± 0.3
DBA	×	MOM	8	6	75.0	6	7.5 ± 1.6
DDK	×	Cas	8	7	87.5	13	7.3 ± 0.4[n.s]
B6	×	Cas	7	6	85.7	11	7.2 ± 0.4
CBA	×	Cas	5	4	80.0	9	6.9 ± 0.5

[a] Reciprocal crosses, MOM females × males of DDK (n = 5), B6 (n = 4), and CBA (n = 3) strains, and Cas females × males of DDK (n = 4), B6 (n = 4), and CBA (n = 3) strains, were performed for mating period of 3 months. Among these matings, only one Cas female mated with a CBA male produced a litter of 6 pups.
[b] No. of pregnant females with litter(s) / no. of females mated.
[c] Means ± SEM.
[n.s] No significant difference among the crosses with MOM males or Cas males at a level of $P > 0.05$ (one-way ANOVA followed by post hoc analysis with Scheffe's tests).

Materials and methods

Mouse strains and general care. Laboratory mouse strains used in the present study were C57BL/6Cr (B6), C3H/HeN (C3H), CBA/J (CBA), DBA/2J (DBA), and DDK. B6 was purchased from Shizuoka Laboratory Animal Corporation (Hamamatsu, Japan). C3H and DBA were introduced from the Institute for Laboratory Animal Research, Graduate School of Medicine, Nagoya University. CBA and DDK were obtained from the Laboratory of Animal Behavioral Physiology and Laboratory of Animal Genetics, respectively, Graduate School of Bioagricultural Sciences, Nagoya University. Production colonies were developed with the introduced individuals, and their descendants were used for the present experiments.

The inbred strain MOM was established from Japanese wild mice (*M. m. molossinus*) in the Laboratory of Animal Genetics, Graduate School of Bioagricultural Sciences, Nagoya University (Nishimura et al. 1973). The individuals at 96–98th generations of brother-sister matings were obtained and used for the present study. The strain Cas was established from Philippine wild mice (*M. m. castaneus*) in the Laboratory of Animal Genetics, Graduate School of Bioagricultural Sciences, Nagoya University. Their original ancestors were captured at Los Baños in the Philippines in 1994. The mice at the 16–20th generations of brother-sister matings were obtained and used for the present experiment. (The Cas strain was renamed CASP after the 20th generation).

The general care of mice was the same as described previously (Zhao et al. 2000), and the animal procedures were performed according to the Guidelines for Animal Experimentation of Nagoya University.

Mating procedure and examination of reproductive performance. The female mice were used at the age of 2–7 months, and males were used at the age of 2–9 months. For the matings between the females of laboratory strains and the males of wild mouse-derived strains, a female was caged with a male for 4 to 20 weeks (mean period: 10 weeks). Mating cages were checked daily for a vaginal plug and birth of young. The number of young inclusive of the dead was counted as the litter size at parturition. Each mating gave 1–3 parturitions; the females that showed a vaginal plug at least once but produced no young and showed no increase in body weight for the period of 4 weeks were counted as infertile after being confirmed to be nonpregnant by dissection. As for the reciprocal matings, a female of the wild mouse-derived strains and a male of the laboratory strains were kept in a cage for a period of three months.

Microsatellite markers and genotype determination. Individuals obtained from backcrosses (N_2 offspring) were genotyped for the *Om* locus. The genotypes were determined by means of three microsatellite markers closely linked to the *Om* locus on Chr 11—*D11Mit247*, *D11Mit66*, and *D11Mit36* (MGD 2001). Genomic DNA was prepared from tails of 12-day-old mice. Oligonucleotide primers for the markers were synthesized by Amersham Pharmacia Biotech, Inc. (Tokyo, Japan). PCR amplification of microsatellite markers was performed according to the procedure of Routman and Cheverud (1994) in 96-well microtitration plates on a Model PC801 machine (ASTEC Company, Fukuoka, Japan). The PCR products were subjected to electrophoresis on 3.5% agarose gels (2% MetaPhor + 1.5% Seakem LE, FMC, Rockland, Maine) in 1 × TBE buffer with 100 V for 1–1.5 h. After electrophoresis, the gels were stained with ethidium bromide and photographed under UV light.

Statistical analysis. Statistical analysis of differences in means among groups was performed by one-way ANOVA, which was followed by post hoc analysis with Scheffe's test. Fitness test for the segregation ratio was performed by the chi-square test. A significance threshold level of $P > 0.05$ was taken as agreement with the expected ratio.

Results

Fertility of DDK females mated with MOM and Cas males. Table 1 shows the reproductive performance in intersubspecific crosses between the females of laboratory strains and the males of MOM and Cas strains. The parturition rates (number of females that produced one or more litters/number of females mated) can be regarded as normal in all crosses (from 75% in DBA females × MOM males to 100% in CBA females × MOM males). The average litter size in DDK females × MOM males was 7.8 ± 0.4, which is comparable to that shown by the females of the other four strains: B6 (9.8 ± 0.7), C3H (9.7 ± 0.6), CBA (7.7 ± 0.3), and DBA (7.5 ± 1.6) ($P > 0.05$). The average litter size in DDK females × Cas males was 7.3 ± 0.4, which is also similar to that exhibited by the females of other two strains: B6 (7.2 ± 0.4) and CBA (6.9 ± 0.5) ($P > 0.05$). These results indicate that no incompatibilities exist among oocytes of the laboratory strains, including B6 and DDK, and sperm of MOM and Cas strains. In contrast, MOM and Cas females were mated with DDK, B6, and CBA males for a mating period of three months. As expected from the generally known fact that wild-derived female mice cannot be successfully mated with males of laboratory strains (Moriwaki et al. 1994), among 23 pairs, only one Cas female mated with a CBA male produced a litter of 6 pups.

Fertility of F_1 mice. Table 2 shows the reproductive performance of the four kinds of F_1 mice mated with DDK, B6, C3H, and MOM strains. The average litter size in DDK females × F_1 (DDK♀ × MOM♂) males was significantly higher than those in other three types of semi-fertile crosses, DDK females × F_1 (B6♀ × DDK♂) males, DDK females × F_1, (B6♀ × MOM♂) males, and DDK females × F_1 (C3H♀ ×

Table 2. Reproductive performance of four kinds of F_1 mice mated with DDK, C57BL/6Cr (B6), C3H/HeN (C3H), and MOM strains.

Types of crosses			No. of females mated	No. of females with litter(s)	Parturition rate (%)	No. of litters born	Average litter size
Dam		Sire					
DDK	×	F_1(B6♀ × DDK♂)	12	11	91.7	24	4.5 ± 0.3[a]
DDK	×	F_1(DDK♀ × MOM♂)	10	9	90.0	18	7.9 ± 0.4[b]
DDK	×	F_1(B6♀ × MOM♂)	10	8	80.0	17	5.1 ± 0.5[a]
DDK	×	F_1(C3H♀ × MOM♂)	4	3	75.0	6	4.8 ± 0.5[a]
B6	×	F_1(B6♀ × MOM♂)	8	8	100.0	15	8.6 ± 0.6[b]
B6	×	F_1(DDK♀ × MOM♂)	7	6	85.7	10	7.3 ± 0.7[b]
C3H	×	F_1(CBH♀ × MOM♂)	4	4	100.0	7	8.0 ± 0.5[b]
F_1(B6♀ × DDK♂)	×	B6	10	10	100.0	25	4.6 ± 0.3[a]
F_1 (DDK♀ × MOM♂)	×	MOM*	9	7	77.8	14	7.2 ± 0.4[b]
F_1(B6♀ × MOM♂)	×	MOM*	8	6	75.0	12	7.1 ± 0.5[b]

* Reciprocal crosses, MOM females × F_1 (DDK♀ × MOM♂) males (n = 7) and F_1 (B6♀ × MOM♂) males (n = 3) were also performed for the mating period of 3 months. Two MOM females mated with F_1 (DDK♀ × MOM♂) males produced a litter of 3 pups, respectively.
[a,b] Values with different superscripts denote significant difference at a level of $P < 0.05$ (one-way ANOVA followed by post hoc analysis with Scheffe's tests) among the crosses with F_1 males or those with F_1 females.
Other footnotes are the same as in Table 1.

Table 3. Reproductive performance of four kinds of F_1 mice mated with DDK, C57BL/6Cr (B6), CBA/J (CBA), and Cas strains.

Types of crosses			No. of females mated	No. of females with litter(s)	Parturition rate (%)	No. of litters born	Average litter size
Dam		Sire					
DDK	×	F_1 (B6♀ × DDK♂)	12	11	91.7	24	4.5 ± 0.3[a]
DDK	×	F_1 (DDK♀ × Cas♂)	11	9	81.8	19	7.2 ± 0.3[b]
DDK	×	F_1 (B6♀ × Cas♂)	14	13	92.9	22	4.5 ± 0.4[a]
DDK	×	F_1 (CBA♀ × Cas♂)	9	7	77.8	10	5.0 ± 0.5[a]
B6	×	F_1 (B6♀ × Cas♂)	10	8	80.0	13	7.9 ± 0.4[b]
B6	×	F_1 (DDK♀ × Cas♂)	8	7	87.5	9	7.6 ± 0.7[b]
CBA	×	F_1 (CBA♀ × Cas♂)	4	4	100.0	7	5.6 ± 0.6[ab]
F_1 (B6♀ × DDK♂)	×	B6	10	10	100.0	25	4.6 ± 0.3[a]
F_1 (DDK♀ × Cas♂)	×	Cas*	7	6	85.7	8	7.1 ± 0.6[b]
F_1 (B6♀ × Cas♂)	×	Cas*	7	6	85.7	9	7.3 ± 0.4[b]

* Reciprocal crosses, Cas females × F_1 (DDK♀ × Cas♂) males (n = 3) and F_1 (B6♀ × Cas♂) males (n = 2) were also performed for the mating period of 3 months, but no offspring were produced from these crosses.
Other footnotes are the same in Tables 1 and 2.

MOM♂) males ($P \ll 0.001$), which give rise to compatible viable embryos and incompatible lethal embryos in an equal ratio. Furthermore, this value does not significantly differ from those in the three types of normal crosses, B6 females × F_1 (B6♀ × MOM♂) males, B6 females × (DDK♀ × MOM♂) males, and C3H females × F_1 (C3H♀ × MOM♂) males ($P > 0.05$), all of which give rise to compatible viable embryos only. These results show that no incompatibility exits between DDK oocytes and the MOM type sperm factor. In addition, the average litter size of F_1 (DDK♀ × MOM♂) females × MOM males is comparable to that of F_1 (B6♀ × MOM♂) females × MOM males ($P > 0.05$), which is considered to show normal fertility; furthermore, it is significantly higher than that in another type of semi-fertile cross F_1 (B6♀ × DDK♂) females × B6 males ($P \ll 0.001$), fertility of which is reduced to a half owing to a 50% incidence of incompatible lethal embryos. These results show that no incompatibility exits between the DDK type oocyte factor and MOM sperm.

Table 3 shows the reproductive performance of four kinds of F_1 mice mated with DDK, B6, CBA, and Cas strains. The average litter size in DDK females × F_1 (DDK♀ × Cas♂) males was significantly higher than the sizes in the three types of semi-fertile crosses, DDK females × F_1 (B6♀ × DDK♂) males ($P \ll 0.001$), DDK females × F_1 (B6♀ × Cas♂) males ($P < 0.001$), and DDK females × F_1 (CBA♀ × Cas♂) males ($P > 0.05$). Furthermore, this value is comparable to those in the three types of normal crosses, B6 females × F_1 (B6♀ × Cas♂) males, B6 females × F_1 (DDK♀ × Cas♂) males, and CBA females × F_1 (CBA♀ × Cas♂) males ($P > 0.05$). In contrast, the average litter size in F_1 (DDK♀ × Cas♂) females × Cas males was also significantly higher than that in the semi-fertile cross F_1 (B6♀ × DDK♂) females × B6 males ($P < 0.001$), and comparable to that in the normal cross F_1 (B6♀ × Cas♂) females × Cas males ($P > 0.05$). These results confirm that no incompatibility exists between DDK type oocyte factor and Cas sperm.

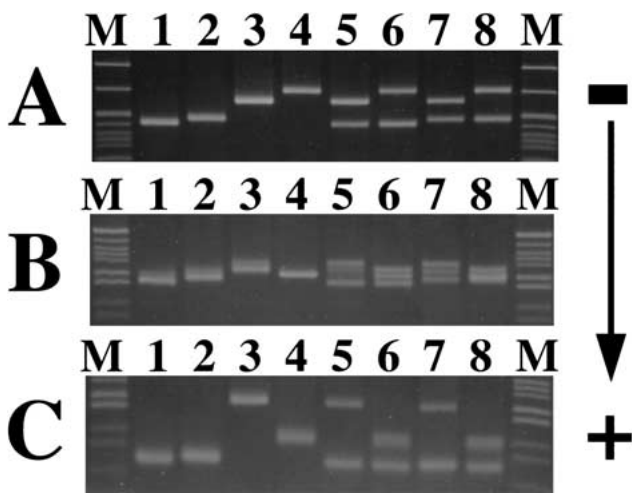

Fig. 1. Detection of three microsatellite markers (A: *D11Mit36*; B: *D11Mit66*; C: *D11Mit247*) on mouse Chr 11 linked to *ovum mutant* locus. M, molecular size marker (pBR322 DNA-*Msp*I digest); 1, DDK; 2, B6; 3, MOM; 4, Cas; 5, F_1 (DDK/MOM); 6, F_1 (DDK/Cas); 7, F_1 (B6/MOM); and 8, F_1 (B6/Cas). Four strains and their F_1 hybrids are distinguishable for all three markers, and the DNA sizes of DDK, B6, MOM, and Cas strains are 220, 236, 283, and 315 bp for *D11Mit36*; 150, 156, 171, and 163 bp for *D11Mit66*; and 96, 99, 158, and 122 bp for *D11Mit247*, respectively.

Table 4. Transmissioni of Om^b, Om^d, Om^M, and Om^C alleles at *ovum mutant* (*Om*) locus through heterozygous F_1 males.[a]

Types of crosses			Genotypes of F_1 males	No. of N_2 examined	Transmission ratios observed in N_2 (%)[b]	Transmission ratios expected (%)[b]	Chi-square value	*P*
Dam		Sire						
DDK	×	F_1 (B6 × DDK)[c]	Om^b/Om^d	115	21/94 (18.3/81.7)	16.1/98.9 (14/86)	1.734	> 0.10
DDK	×	F_1 (DDK♀ × MOM♂)	Om^d/Om^M	101	44/57 (43.6/56.4)	50.5/50.5 (50/50)	1.674	> 0.10
DDK	×	F_1 (B6♀ × MOM♂)	Om^b/Om^M	54	9/45 (16.7/83.3)	7.6/46.4 (14/86)	0.322	> 0.50
B6	×	F_1 (B6♀ × MOM♂)	Om^b/Om^M	94	44/50 (46.8/53.2)	47.0/47.0 (50/50)	0.383	> 0.50
DDK	×	F_1 (DDK♀ × Cas♂)	Om^d/Om^C	99	54/45 (54.5/45.5)	49.5/49.5 (50/50)	0.827	> 0.25
DDK	×	F_1 (B6♀ × Cas♂)	Om^b/Om^C	62	10/52 (16.1/83.9)	8.7/53.3 (14/86)	0.226	> 0.50
B6	×	F_1 (B6♀ × Cas♂)	Om^b/Om^C	92	43/49 (46.7/53.3)	46.0/46.0 (50/50)	0.391	> 0.50

[a] Genotypes for *Om* locus of backcross (N_2) offspring were determined by PCR method with three microsatellite markers closely linked to *Om* locus (*D11Mit* 247, 66, and 36). Data obtained with the three markers were almost identical to one another (see text), and only the data of *D11Mit* 36 are shown in this table.
[b] Observed number of transmitted alleles are shown in the same order as indicated in genotypes of F_1 males. Expected ratios for DDK females × F_1(B6/DDK), F_1 (B6♀ × MOM♂), and F_1 (B6♀ × Cas♂) males are calculated on the basis of 14% transmission ratio for Om^b, and those for other crosses are calculated on the basis of 50% transmission ratio for Om^M or Om^C (see text).
[c] F_1 males from the reciprocal crosses, B6♀ × DDK♀ and DDK♀ × B6♂, were used for matings, and 55 offspring from the former and 60 offspring from the latter were genotyped. No significant difference was seen between the two groups, and the data are combined.

Transmission of Om alleles from F_1 males to backcross (N_2) offspring. It was shown by Pardo-Manuel de Villena et al. (2000) that the offspring from the backcross DDK females × F_1 (B6♀ × DDK♂) males segregated into 14% heterozygotes (Om^b/Om^d) and 86% homozygotes (Om^d/Om^d). We have investigated whether a similar transmission ratio is observed also in the backcrosses involving F_1 (DDK♀ × MOM♂) males and F_1 (DDK♀ × Cas♂) males by means of the three microsatellite markers closely linked to the *Om* locus, *D11Mit247*, *D11Mit66*, and *D11Mit36*. Figure 1 shows that the microsatellite markers amplified by the three primers can be distinguished from one another among four strains, DDK, B6, MOM, and Cas, and their F_1 hybrids. Genotypings of backcross (N_2) offspring by the three markers showed almost the same results, and the recombination rates between these marker loci in the three kinds of F_1 males were about 1–2% (data not shown).

Genotypings of the backcross (N_2) offspring produced by DDK and B6 females mated with five kinds of F_1 males are shown in Table 4. Expected segregation ratios among the offspring from DDK females mated with three kinds of F_1 males—F_1 (B6♀ × DDK♂), F_1 (B6♀ × MOM♂), and F_1 (B6♀ × Cas♂)—were calculated by referring to the above report, i.e., 14% transmission for Om^b allele and 86% transmission for Om^d, Om^M, and Om^C alleles, because Om^M and Om^C alleles have been found to function identically as the Om^d allele in respect to sperm factor. Expected segregation ratios among the offspring from other crosses were calculated on the basis of equal (50%) transmission of each allele. In DDK females × reciprocal F_1 (B6 × DDK) males, the transmission ratio of Om^b:Om^d from F_1 males to N_2 offspring was 18.3%:81.7%, which is not significantly different from the expected 14%:86% ratio ($P > 0.1$). In the other two types of backcrosses, DDK females × F_1 (B6♀ × MOM♂) males and DDK females × F_1 (B6♀ × Cas♂) males, the transmission ratios of Om^b:Om^M and Om^b:Om^C were 16.7%:83.3% and 16.1%:83.9%, respectively, both of which are comparable to the expected ratio ($P > 0.5$). These results indicate that MOM and Cas type sperm factors are compatible with DDK oocytes. Furthermore, in the two types of backcrosses DDK females × F_1 (DDK♀ × MOM♂) males and DDK females × F_1 (DDK♀ × Cas♂) males, the transmission ratios of Om^d:Om^M and Om^d:Om^C were in agreement with the expected 50%:50% ratio ($P > 0.1$ and $P > 0.25$, respectively), confirming the compatibility between DDK oocytes and MOM and Cas type sperm factors. The transmission ratios of each allele in B6 females × F_1 (B6♀ × MOM♂) males and B6 females × F_1 (B6♀ × Cas♂) males were also in accordance with the expected 50%:50% ratio ($P > 0.5$). Accordingly, it may be concluded that the MOM and Cas type sperm factors are compatible with DDK and B6 oocytes.

Fertility of F_1 females mated with DDK and B6 males. In order to determine whether the MOM and Cas oocytes are compatible with DDK and B6 sperm, the mating experiments were performed with DDK and B6 males and four kinds of F_1 females (DDK or B6 females × MOM or Cas males), and the results are shown in Table 5. Two kinds of F_1 females (DDK♀ × MOM♂ and B6♀ × MOM♂) mated with DDK males showed significantly smaller litter size (6.6 ± 0.3 and 6.6 ± 0.6, respectively) compared with F_1 (B6♀ × DDK♂) females mated with DDK males (9.0 ± 0.6, $P < 0.05$). But these values are comparable with those in crosses F_1 females (DDK♀ × MOM♂ and B6♀ × MOM♂) mated with MOM males (7.2 ± 0.4 and 7.1 ± 0.5 respectively: Table 2). There is a possibility that a smaller number of oocytes may be ovulated in the MOM-derived F_1 females compared with the F_1 (B6♀ × DDK♂) females, since the average litter size in the MOM strain is 4.4 ± 0.4 (N = 7) and that in the B6 and DDK strains is 7.8 ± 0.3 (N = 18) and 7.1 ± 0.4 (N = 24), respectively. Taking these points into consideration, it is inferred that there is no incompatibility between the MOM type oocyte factor and DDK sperm. The average litter size of F_1 (B6♀ × MOM♂) females × B6 males is comparable with that of F_1 (B6♀ × MOM♂) females × MOM males (6.3 ± 0.5 vs. 7.1 ± 0.5, $P > 0.05$), indicating that there is no incompatibility between MOM type oocyte factor and B6 sperm. On the other hand, the three kinds of F_1 (DDK♀ × Cas♂, B6♀ × Cas♂, and CBA♀ × Cas♂) females mated with DDK males showed slightly larger litter size compared with the F_1 females derived from MOM males. This is thought to reflect a large number of oocytes ovulated in the F_1 females derived from Cas males, because the average litter size in the Cas strain is slightly larger (5.3 ± 0.5, N = 10) than that of the MOM strain. The litter sizes in these three types of crosses are comparable to those in the two crosses, F_1 (DDK♀ × Cas♂) females × Cas males and F_1 (B6♀ × Cas♂) females × Cas males (Table 3); furthermore, they do not differ significantly from that in the cross F_1 (B6 ♀ × DDK♂) females × DDK males showing normal fertility ($P > 0.05$). Therefore, it is inferred that no incompatibility exists between Cas type oocyte factor and DDK sperm.

In F_1 (DDK♀ × MOM♂) females × B6 males and F_1 (DDK ♀ × Cas♂) females × B6 males, the average litter size was comparable ($P > 0.05$), but was reduced to about half that of normal cross F_1 (B6 ♀ × DDK ♂) females × DDK males. This reduction in litter size is thought to be caused by the incompatibility attributed to DDK type oocyte factor and B6 sperm. Therefore, it is presumed that the oocyte factor

Table 5. Reproductive performance of six kinds of F_1 females mated with males of DDK or C57BL/6Cr (B6) strains.

Types of crosses			No. of females mated	No. of females with litter(s)	Parturition rate (%)	No. of litters born	Average litter size
Dam		Sire					
F_1 (B6♀ × DDK♂)	×	DDK	12	12	100.0	19	9.0 ± 0.6[a]
F_1 DDK♀ × MOM♂)	×	DDK	10	9	90.0	20	6.6 ± 0.3[b]
F_1 (DDK♀ × MOM♂)	×	DDK	7	7	100.0	10	6.6 ± 0.6[b]
F_1 (DDK♀ × MOM♂)	×	B6	12	10	83.3	18	4.9 ± 0.4[bc]
F_1 (B6♀ × MOM♂)	×	B6	8	7	87.5	11	6.3 ± 0.5[bc]
F_1 (DDK♀ × Cas♂)	×	DDK	9	8	88.9	13	7.6 ± 0.4[ab]
F_1 (B6♀ × Cas♂)	×	DDK	5	5	100.0	10	8.9 ± 0.6[a]
F_1 (CBA♀ × Cas♂)	×	DDK	6	6	100.0	12	7.0 ± 0.3[ab]
F_1 (DDK♀ × Cas♂)	×	B6	10	9	90.0	13	5.3 ± 0.5[bc]
F_1 (B6♀ × Cas♂)	×	B6	9	8	88.9	14	4.3 ± 0.4[c]

For the footnotes, refer to Table 1.

Table 6. Transmission of Om^b, Om^d, Om^M, and Om^C alleles at *ovum mutant* (*Om*) locus through heterozygous F_1 females.[a]

Types of crosses			Genotypes of F_1 females	No. of N_2 examined	Transmission ratios observed in N_2 (%)[b]	Transmission ratios expected (%)[c]	Chi-square value	*P*
Dam		Sire						
F_1 (B6♀ × DDK♂)	×	DDK	Om^b/Om^d	24	11/13 (45.8/54.2)	12.0/12.0 (50/50)	0.167	> 0.50
F_1 (DDK♀ × B6♂)	×	DDK	Om^b/Om^d	42	23/19 (54.8/45.2)	21.0/21.0 (50/50)	0.381	> 0.50
F_1 (B6♀ × DDK♂)	×	B6	Om^b/Om^d	66	26/40 (39.4/60.6)	26.4/39.6 (40/60)	0.001	> 0.90
F_1 (DDK♀ × B6♂)	×	B6	Om^b/Om^d	37	19/18 (51.4/48.6)	14.8/22.2 (40/60)	1.987	> 0.10
F_1 (DDK♀ × MOM♂)	×	DDK	Om^d/Om^M	36	17/19 (47.2/52.8)	18.0/18.0 (50/50)	0.111	> 0.50
F_1 (B6♀ × MOM♂)	×	B6	Om^b/Om^M	25	13/12 (52.0/48.0)	12.5/12.5 (50/50)	0.040	> 0.75
F_1 (DDK♀ × Cas♂)	×	DDK	Om^d/Om^C	26	14/12 (53.8/46.2)	13.0/13.0 (50/50)	0.154	> 0.50
F_1 (B6♀ × Cas♂)	×	B6	Om^b/Om^C	34	15/19 (44.1/55.9)	17.0/17.0 (50/50)	0.471	> 0.25

[a] Genotypes for the *Om* locus of backcross (N_2) offspring were determined by *D11Mit36*.
[b] Observed numbers of transmitted alleles are shown in the same order as indicated in genotypes of F_1 females.
[c] Expected ratios are calculated on the basis of 50% transmission ratio for each allele except for those in two kinds of crosses, F_1 (B6♀ × DDK♂) females × B6 males and F_1 (DDK♀ × B6♂) females × B6 males, which were calculated on the basis of 40%:60% transmission ratio for Om^b:Om^d (see text).
Other footnotes are the same as in Table 4.

controlled by the Om^M or Om^C allele is compatible with the sperm factor controlled by the Om^b allele; in other words, MOM and Cas oocytes are compatible with B6 sperm. However, the average litter size of F_1 (B6♀ × Cas♂) females × B6 males was also reduced to about half that of F_1 (B6♀ × Cas♂) females × DDK males, suggesting that the oocyte factor controlled by the Om^C allele is incompatible with B6 sperm. This is contradictory to the above presumption that the Cas type oocyte factor is compatible with B6 sperm.

Transmission of Om alleles from F_1 females to backcross (N_2) offspring. In search of a clue to interpret the above contradiction, transmission of *Om* alleles from F_1 females to the backcross (N_2) offspring was investigated. In this experiment, N_2 offspring were genotyped only for the *D11Mit36* microsatellite marker; the results are shown in Table 6. Following the report on the maternal transmission ratio distortion (TRD) at *Om* locus (Pardo-Manuel de Villena et al. 1996, 1997), the expected transmission ratio of Om^d and Om^b was taken as 40%:60% for two kinds of crosses: F_1 (B6 ♀ × DDK♂) females × B6 males and F_1 (DDK ♀ × B6♂) females × B6 males. The expected transmission ratio for other crosses was taken as 50%:50% for each allele. As expected, F_1 (B6♀ × DDK♂) females × B6 males showed a transmission ratio of 39.4%:60.6% ($P > 0.90$). The transmission in F_1 (DDK ♀ × B6♂) females × B6 males was also in agreement with the expected ratio ($P > 0.10$). In all other crosses, the transmission ratios were in agreement with 50%:50% ratio ($P > 0.25$). The equal transmission ratio was observed also in the backcross F_1 (B6♀ × Cas♂) females × B6 males, in which litter size was reduced to about half that of F_1 (B6♀ × Cas♂) females × DDK males. Although the Om^C allele was transmitted slightly more than the Om^b allele, these results have merely confirmed that the transmission ratio of *Om* alleles from the F_1 females to the N_2 offspring is basically independent of the incompatibility between oocyte and sperm factors, and it seems to be difficult to find a clue to solve the above contradiction.

Discussion

The present study demonstrated that the female mice of several laboratory strains, including B6 and DDK, showed normal fertility when mated with males of MOM and Cas strains, which were originated from Japanese wild mice (*M. m. molossinus*) and Philippine wild mice (*M. m. castaneus*), respectively; in other words, B6 and DDK oocytes are compatible with MOM and Cas sperm. Moreover, the mating experiments involving F_1 females have presented the following two suggestions: (1) MOM oocytes are compatible with B6 and DDK sperm; (2) Cas oocytes are compatible with DDK sperm. However, the relation between Cas oocytes and B6 sperm has not been made clear.

The DDK strain was established in Japan from so-called German mice that were introduced from Germany, and the strain is regarded as European mice, having the genes derived from *M. m. domesticus* subspecies in large quantities (Goto et al. 1979, 1982; Festing 1994). It is agreed that some of the Japanese fancy mice were introduced into Europe and crossed with European mice. Such mixed mouse populations formed the foundation stocks from which European and North American laboratory strains were established (Goto et al. 1982; Nishioka 1995; Koide et al. 1998). *M. m. molossinus* is regarded as the hybridized population of *M. m. castaneus* and *M. m. musculus* (Yonekawa et al. 1988; Bonhomme et al. 1989). From the results of the present study, the following assumption may be made. The DDK strain carries the mutated allele at the *Om* locus, and the oocyte factor controlled by the

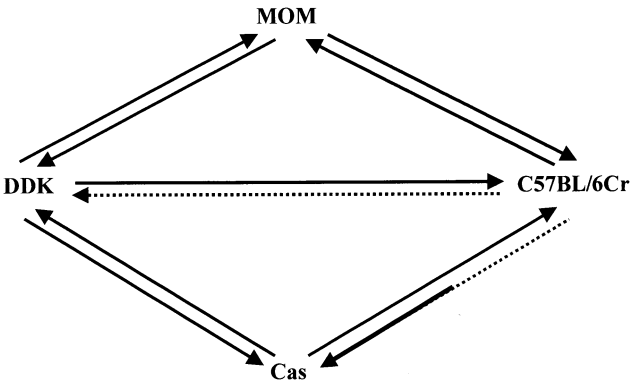

Fig. 2. Relationships between oocyte and sperm factors controlled by the alleles at *ovum mutant* locus among DDK, C57BL/6Cr, MOM, and Cas strains. Arrows indicate the direction from sperm to oocytes, and the compatibility is shown as follows: —, compatible; ·····, incompatible; ···—, unknown.

mutated allele (DDK type oocyte factor) is incompatible with the sperm carrying a domesticus type normal allele, but compatible with the sperm carrying molossinus and castaneus type alleles. On the contrary, the sperm carrying the mutated allele are compatible with all four types of oocytes. The exception to this assumption may be the observation that the DDK females showed reduced fertility when mated with the males of the NC and KK strains (Wakasugi et al. 1967). These two inbred strains are believed to be established from Japanese mice (Festing 1994), but there is a possibility that they may have originated from the European mice with mixed origins mentioned above, since it has been found that NC is more closely related to B6 than to MOM when judged by the degree of differences in microsatellite markers (Wada et al. 2000). In this context, it would be interesting to perform the mating experiments with the DDK strain and *M. m. musculus*.

The relationships of the oocyte and sperm factors among DDK, B6, MOM, and Cas strains are schematically summarized in Fig 2. In spite of the unidirectional incompatibility between the DDK and B6 strains, the DDK and MOM strains are mutually compatible, and the B6 and MOM strains are also mutually compatible. This interrelation among DDK, B6, and MOM is difficult to interpret on the bases of genetics and molecular-developmental biology. The embryonic death in the DDK syndrome is caused by the incompatibility between the oocyte and sperm factors, and the lethal embryo is characterized by the failure of blastocyst formation, i.e., trophectoderm differentiation. Therefore, the interaction between oocyte and sperm factors beginning at fertilization is thought to trigger the mechanism controlling differentiation of trophectoderm, and the clue to the above problem might be obtained through the investigation of the molecular mechanisms regulating trophectoderm differentiation.

The semi-fertility of F_1 (DDK♀ × Cas♂) females mated with B6 males is thought to be the incompatibility between DDK type oocyte factor and B6 sperm and suggests that the Cas type oocyte factor may be compatible with B6 sperm. In contrast, the contradictory presumption that Cas type oocyte factor may be incompatible with B6 sperm was given by the following two observations: (1) the litter size of F_1 (B6♀ × Cas♂) females × B6 males was reduced to about half of the normal; (2) Om^b and Om^C alleles were equally transmitted from the F_1 females to the backcross offspring in this cross. B6 strain has been shown to possess a modifier gene(s) that reduces the fertility of heterozygous (On^b/Om^d) females mated with B6 males (Pardo-Manuel de Villena et al. 1999; Zhao et al. 2000). However, it is impossible to explain the above contradictory observation by this type of modifier gene, which is supposed to direct the heterozygous (Om^b/om^d) females to produce more oocytes with DDK type oocyte factor than those with B6 type oocyte factor. Provided that the Cas type oocyte factor is incompatible with B6 sperm, the cross F_1 (DDK♀ × Cas♂) females × B6 males must be completely infertile; in other words, the oocytes produced by the F_1 (DDK♀ × Cas♂) females are either DDK type or Cas type, and both of them are incompatible with B6 sperm. Further information is needed to clarify the relation between the Cas type oocyte factor and B6 sperm, and as a next step it is necessary to investigate when and how embryonic or fetal losses occur in F_1 (B6♀ × Cas♂) females × B6 males. It is also important to examine the fertility of the homozygous (Om^C/Om^C) backcross females when they are mated with B6 males.

MOM and Cas strains would be useful for further investigation of the DDK syndrome, although many aspects remain to be clarified. The DDK strain may be used efficiently for experiments based on the intersubspecific hybridization with MOM and Cas strains, and, therefore, the DDK strain can be used more widely for various studies in the future. It has been used so far in specific researches such as a pregnancy block by male pheromones (Chung et al. 1997, 1999) and expression of D14 egg-specific polypeptide translated from maternal mRNA (Richoux et al. 1991), owing to the limitation from the DDK syndrome.

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