Major genes control hormone-induced ovulation rate in mice

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Summary. The present study examined the magnitude of genetic variation, mode of inheritance and number of loci controlling major genetic differences in hormone-induced ovulation rate in mice. Mice were injected with 5 i.u. PMSG at 28 days of age and 5 i.u. hCG at 30 days, and hormone-induced ovulation rate was determined from counts of oviducal eggs in cumulus the next morning. Six-fold genetic differences in induced ovulation rate were detected amongst strains, ranging from a low mean ($\pm$ s.e.) value of 8.8 ($\pm$ 0.9) in A/J up to 53.5 ($\pm$ 2.2) in C57BL/6J. The number of ova differed significantly amongst strains and amongst F_1 crosses ($P < 0.0001$): 70% of the variation in hormone-induced ovulation rate was amongst strains. Of 9 F_1 crosses examined, 4 showed positive heterosis, 3 showed no heterosis and 2 showed negative heterosis for hormone-induced ovulation rate. Analysis of parental, F_1 and F_2 generations revealed that the induced ovulation rate of A/J and C57BL/6J mice differed due to the action of about 3 or 4 loci, and A.SW/SnJ and SJL/J mice differed due to the action of about 2 to 3 loci. Analysis of recombinant inbred strains formed from A/J and C57BL/6J confirmed that these strains differed due to the action of a small number of loci. This study demonstrates the existence of a small number of major genes controlling hormone-induced ovulation rate in young mice.

Keywords: genes; ovulation rate; mice; heterosis

Introduction

The number of eggs ovulated at a given oestrus, or ovulation rate, is a function of the serum concentration of gonadotrophins and the ovarian sensitivity, or responsiveness to gonadotrophins (Spearow, 1980; Webb & Gauld, 1985). Laboratory and farm animal species show a considerable amount of genetic variation in the ovarian responsiveness to exogenous gonadotrophins (Fowler & Edwards, 1957; McLaren, 1962; Bradford, 1969; Land & Falconer, 1969; Bradford *et al.*, 1971; Bindon & Pennycuik, 1974; Durrant *et al.*, 1980; Spearow, 1980; Spearow & Bradford, 1983). Unfortunately, the magnitude and nature of genetic variation in hormone-induced ovulation rate are not completely understood. Previous studies have been limited by the lack of highly inbred lines or well defined major breed differences in hormone-induced ovulation rate in most species. Furthermore, highly inbred strains which do differ dramatically in hormone-induced ovulation rate have not been used to determine whether genes with large effects are involved.

The objectives of the present study were to determine (1) the magnitude of the genetic variation in hormone-induced ovulation rate by screening genetically defined, highly inbred strains of mice, (2) the nature of the genetic variation of hormone-induced ovulation rate in mice by examining the

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responses of recombinant inbred strains and F_1 crosses of these strains and, (3) the number of loci controlling these genetic differences in induced ovulation rate by comparing the performance of divergent inbred strains with their F_1 and F_2 crosses.

Materials and Methods

Lines and husbandry of mice. Highly inbred mouse strains A/J, AKR/J, CBA/J, SEC/1ReJ, SWR/J, DBA/2J, BALB/cJ, 129/SvJ, C3H/HeJ, SM/J, C57L/J, C57BL/10J, SJL/J and C57BL/6J, and an *H-2* congenic strain, A.Sw/SnJ, were obtained from the Jackson Laboratory (Bar Harbor, Maine, U.S.A.). Strains S15 and C17 were produced by more than 20 generations of full sib mating from the litter size line (Line S1) and control line (Line C) in the laboratory of Dr G. E. Bradford (University of California at Davis). The development of strains S15 and C17 has been previously described by Spearow & Bradford (1983). Several crosses amongst the above strains were also used. The recombinant inbred strains (AB5, AB13, AB15, AB17 and BA14) were developed by Dr Murriel Nesbitt by inbreeding from crosses of A/J and C57BL/6J. These recombinant inbred strains were obtained from Dr Robert P. Erickson. Mice compared in each experiment were in groups that were as contemporary as possible. The performance of inbred strains and crosses was examined in an overlapping manner as mice became available from the breeding operation. Lights were on from 07:00 to 21:00 h, and the temperature was maintained at 22–24°C. Mouse chow (Ralston Purina Company No. 5015) with an approximate analysis of 17% crude protein, 11% crude fat and 3% crude fibre was fed *ad libitum*.

Hormone-induced ovulation rate. PMSG was obtained from the hormone distribution program, NIADDK, National Institutes of Child Health and Human Development, Bethesda, MD. Human chorionic gonadotrophin with an activity of 2700 i.u./mg was obtained from Hypo Lab, Coinsins, Switzerland. Preliminary experiments were conducted to optimize the dose of gonadotrophins and the timing of their administration to mice for the detection of genetic variation. The protocol selected for all experiments described in this study consisted of (1) injecting 5 i.u. PMSG subcutaneously (s.c.) in 50 μ l 0.02 M-NaPO₄, 0.14 M-NaCl, 0.1% gelatin, pH 7.2 (PBS-gel) at 28 days of age, (2) injecting 5 i.u. hCG in 50 μ l PBS-gel 48–52 h later (30 days of age) and (3) determining the number of ova with and without cumulus in the oviduct 16–22 h later (31 days of age). To do the latter, mice were weighed, killed by cervical dislocation and the number of freshly ovulated ova in cumulus (ova) and the number of ova without cumulus (old ova) were determined with the aid of a dissecting microscope. Lyophilysed samples of the same lot of PMSG and hCG were used for all experiments.

Preliminary experiments showed that any eggs ovulated by the first dose of gonadotrophins on Day 28 were in cumulus 16–22 h later on Day 29 but were all out of cumulus by Day 31. Since by Day 31 these eggs had been present in the oviduct for 2 days and were without cumulus, these ova were referred to as 'old ova'. Preliminary experiments also showed that freshly ovulated eggs were all in cumulus 16–22 h after hCG. Only eggs in cumulus on Day 31 were considered for calculation of hormone-induced ovulation rate.

In Exp. 1, 16 strains of mice were screened to determine the magnitude of genetic variation in hormone-induced ovulation rate. In Exp. 2 the induced ovulation rate of several F_1 strain combinations was examined to determine the mode of inheritance of induced ovulation rate. Experiments 3 and 4 examined induced ovulation rate of several inbred strains and their F_1 and F_2 crosses to determine the number of loci mediating the observed genetic differences. In Exp. 5, the induced ovulation rate of recombinant inbred strains of A/J and C57BL/6J were examined to confirm that the hormone-induced ovulation rate of these strains of mice differed due to the action of a small number of loci.

Statistical analysis. Data from Exps 1, 2 and 5 were analysed with one-way analyses of variance, covariance and multiple range tests. Since no dramatic differences in induced ovulation rate were readily apparent amongst the reciprocal crosses of the strains in Exp. 2, they were combined. In Exp. 2, since inbred strains were unrelated, the variance of the mid-parental average was estimated as $1/4 \text{ variance}_{p_1} + 1/4 \text{ variance}_{p_2}$. The variance of the difference between the F_1 mean and the mid-parental mean was calculated as $(\text{variance}_{F_1}/n - 1) + (\text{variance}_{\text{midparental average}}/n - 1)$ (Snedecor & Cochran, 1980). Significance of deviation of F_1 from the mid-parental mean was determined by *t* test. Since the variances within strains for induced ovulation rate were not equal, ANOVAs were conducted on untransformed data using the Brown–Forsythe test (Brown & Forsythe, 1974; Dixon, 1983) because it does not require the assumption of homogeneity of within-group variances. Furthermore log and square-root transformations were performed on the data. While a log transformation slightly improved the homogeneity of variances for hormone-induced ovulation rate in some experiments, log correction was too extreme. Square-root transformation resulted in a much better homogeneity of variances amongst strains and crosses. Therefore data were also analysed after square-root transformation.

The number of loci (*N*) by which strains A/J and C57BL/6J (Exp. 3) and strains A.SW/SnJ and SJL/J (Exp. 4) differed with respect to hormone-induced ovulation rate was determined by the method of Wright (1968) for strains which are at extremes. With no dominance, *N* is equal to the segregation ratio (*S*), where:

$$S = \frac{(\text{mean}_{\text{high strain}} - \text{mean}_{\text{low strain}})^2}{8 (\text{variance}_{F_2} - \text{variance}_{\text{environment}})}$$

The environmental variance was estimated as that of the F_1 or that of the pooled variance within both parents and the F_1 . With dominance $N = [1.5 - 2h(1 - h)]S$. Where *h* was estimated as $a_1/(a_1 + a_2)$. The number of loci by which

these strains of mice differed with respect to hormone-induced ovulation rate was determined both with and without square-root transformation of the data and utilizing the variance of the F_1 and using the pooled variance as estimates of the environmental variance.

Results

Experiment 1: hormone-induced ovulation rate of inbred strains

Figure 1 shows the number of ova ovulated by mice of 16 inbred strains in response to PMSG and hCG. Strains have been arranged in order of increasing hormone-induced ovulation rate. A tremendous amount of genetic variation in induced ovulation rate was detected. Some strains such as A/J and AKR/J ovulated a mean of about 9 eggs, while other strains such as SJL/J and C57BL/6J ovulated means of 47 and 54 eggs, respectively. The number of ova and square-root transformed ova differed significantly amongst strains ($P < 0.0001$) with 70.5% and 74.8% of the variation in ova and square-root ova being amongst strains, respectively.

Fig. 1. Hormone-induced ovulation rate of mice of 16 inbred strains, i.e. no. of ova found in cumulus in response to 5 i.u. PMSG on Day 28 and 5 i.u. hCG on Day 30. Values are mean $\pm$ s.e.m. for the no. of mice shown.

Body weight of mice at 31 days of age averaged 15.6 g, but there were differences in mean body weight amongst strains ($P < 0.001$). The effect of body weight on hormone-induced ovulation rate was therefore tested. The regression of body weight on hormone-induced ovulation rate (-1.38 ± 0.34 ova per g body weight) was significant ($P < 0.0001$) and differed only marginally amongst strains ($P < 0.1$). However, correction for the effect of body weight did not significantly change the ranking of any of the strains with respect to hormone-induced ovulation rate. After correction for body weight, 72% of the variation in induced ovulation rate was amongst strains.

Strains also differed in the number of old eggs matured by endogenous gonadotrophins and ovulated by the initial dose of PMSG ($P < 0.0001$) (Fig. 2). The number of old ova ovulated by C57BL/6J in response to 5 i.u. PMSG was greater than that of A/J ($P < 0.01$), DBA/2J ($P < 0.01$), 129/SvJ ($P < 0.01$) and SJL/J ($P < 0.1$) mice. Correction for the effect of body weight ($b = 0.53 \pm 0.12$ old ova per g body weight; $P < 0.001$) did not significantly change the ranking of strains for old ova.

Fig. 2. Number of old ova found without cumulus in response to 5 i.u. PMSG on Day 28, and 5 i.u. hCG on Day 30, in mice of different strains. Values are mean $\pm$ s.e.m. for the no. of mice shown.

Table 1. Hormone induced ovulation rate of F_1 mice

F_1 cross	Hormone induced ovulation rate				
	No.	Mean $\pm$ s.e.m.	Mid-parental average	Significance of difference of F_1 from:	
				Mid-parental average	Nearest parent
A/J $\times$ AKR/J F_1	10	17.0 $\pm$ 1.7	8.9	**	**
A/J $\times$ SEC/1ReJ F_1	22	14.0 $\pm$ 1.3	10.5	*	NS
A/J $\times$ DBA/2J F_1	10	23.1 $\pm$ 2.9	15.1	*	NS
A/J $\times$ SJL/J F_1	12	23.8 $\pm$ 1.3	27.9	NS	**
C57BL/6J $\times$ A/J F_1	13	27.2 $\pm$ 2.4	31.1	NS	**
C57BL/6J $\times$ AKR/J F_1	7	35.3 $\pm$ 3.6	31.2	*	**
C57BL/6J $\times$ DBA/2J F_1	27	25.5 $\pm$ 1.9	37.4	**	NS
C57BL/6J $\times$ 129/SvJ F_1	17	34.6 $\pm$ 2.3	40.5	NS	*
C57BL/6J $\times$ SJL/J F_1	16	37.1 $\pm$ 3.4	50.2	**	*

* $P < 0.05$; ** $P < 0.01$; NS $P > 0.05$.

Experiment 2: hormone-induced ovulation rate of F_1 strains of mice

Table 1 shows the hormone-induced ovulation rate of F_1 mice that have been arranged in the order of increasing mid-parental average (i.e. the average of the two parental strains). Significant differences in hormone-induced ovulation rate were found amongst F_1 mice ($P < 0.0001$). After square-root transformation of the data from 9 F_1 crosses, 4 crosses (A/J $\times$ AKR/J F_1 , A/J $\times$ SEC/1ReJ F_1 , A/J $\times$ DBA/2J F_1 , and C57BL/6J $\times$ AKR/J F_1) showed positive heterosis, 3 crosses (A/J $\times$ SJL/J F_1 , C57BL/6J $\times$ A/J F_1 and C57BL/6J $\times$ 129/SvJ F_1) showed no heterosis, and 2 crosses (C57BL/6J $\times$ DBA/2J F_1 and C57BL/6J $\times$ SJL/J F_1) showed negative heterosis (i.e. deviation from the mid-parental mean) for hormone-induced ovulation rate. The A/J $\times$ AKR/J F_1 showed positive heterosis for hormone-induced ovulation rate ($P < 0.001$). Indeed the hormone-induced ovulation rate of this F_1 was almost twice that of either parent. While the A/J $\times$ SEC/1ReJ F_1 shows positive heterosis ($P < 0.05$), the observation that the induced ovulation rate of this F_1 was similar to that of the SEC/1ReJ parent suggests dominance. The C57BL/6J $\times$ A/J F_1 , the A/J $\times$ SJL/J F_1 and the C57BL/6J $\times$ 129/SvJ F_1 showed no heterosis, since after log transformation

Fig. 3. Distribution of hormone-induced ovulation rate of A/J and C57BL/6J strains, and their F_1 and F_2 crosses.

the induced ovulation rate of these F_1 crosses was not significantly different from the mid-parental values. While crosses involving DBA/2J showed heterosis, a closer look revealed that crosses with DBA/2J actually showed complete dominance. Regardless of whether DBA/2J was crossed with the lowest strain, A/J, or the highest strain, C57BL/6J, the induced ovulation rates of the resulting F_1 hybrids were the same as that of the DBA/2J parent. On the average, the F_1 crosses which had A/J as a parent ovulated 2.3 more ova than their mid-parental average. In contrast, F_1 s which had C57BL/6J as a parent ovulated 6.2 eggs less than their mid-parental average.

The body weight of F_1 mice averaged 15.9 g at 31 days of age, body weight at 31 days of age differed amongst F_1 crosses ($P < 0.001$), and the effect of body weight on induced ovulation rate differed significantly amongst F_1 mice ($P < 0.01$).

For each F_1 cross, the mean $\pm$ s.e.m. number of old ova found in the oviduct on Day 31 was 6.8 ± 2.3 for A/J $\times$ AKR/J F_1 ; 7.7 ± 1.6 for A/J $\times$ SEC/1ReJ F_1 ; 4.6 ± 1.4 for A/J $\times$ DBA/2J F_1 ; 2.4 ± 1.3 for A/J $\times$ SJL/J F_1 ; 7.5 ± 1.3 for C57BL/6J $\times$ A/J F_1 ; 6.3 ± 1.5 for C57BL/6J $\times$ AKR/J F_1 ; 7.7 ± 1.0 for C57BL/6J $\times$ DBA/2J F_1 ; 6.4 ± 1.2 for C57BL/6J $\times$ 129/SvJ F_1 ; and 8.3 ± 1.1 for C57BL/6J $\times$ SJL/J F_1 . The number of old ova did not differ significantly amongst F_1 mice ($P = 0.14$), and body weight did not have a significant effect ($P = 0.27$).

Experiment 3: number of loci differing amongst A/J and C57BL/6J mice

The procedure of Wright (1968) was used to determine the number of loci mediating the 6-fold genetic differences between strains with low and high induced ovulation rates. This procedure uses the difference in the mean and variances of parents and their F_1 and F_2 crosses to estimate the number of loci. Figure 3 shows the distribution of the hormone-induced ovulation rate of strains A/J and C57BL/6J and their F_1 and F_2 crosses. The mean $\pm$ s.d. hormone-induced ovulation rates were 8.8 ± 4.0 ($N = 20$) for A/J, 53.5 ± 14.7 ($N = 44$) for C57BL/6J, 32.9 ± 10.0 ($N = 38$) for A/J $\times$ C57BL/6J F_1 and 27.3 ± 13.1 ($N = 113$) for A/J $\times$ C57BL/6J F_2 . The mean $\pm$ s.d. square root transformed hormone-induced ovulation rates were 2.883 ± 0.717 for A/J, 7.235 ± 1.068 for C57BL/6J, 5.671 ± 0.851 for A/J $\times$ C57BL/6J F_1 and 5.090 ± 1.195 for A/J $\times$ C57BL/6J F_2 .

Analysis of the untransformed data revealed estimates of 3.5 loci (using F_1 variance) and 3.96 loci (using the pooled variance) by which A/J and C57BL/6J differ. The same analysis of the square-root transformed data revealed estimates of 3.36 (using F_1 variance) and 3.63 (using the pooled variance) loci by which A/J and C57BL/6J differ. Inclusion of the effect of dominance in a model using the F_1 variance as an estimate of environmental variance resulted in estimates of 3.54 loci (raw data) and 3.49 loci (square root transformed data) by which strain A/J and C57BL/6J mice differ with respect to hormone-induced ovulation rate. Therefore, regardless of the assumptions used, the hormone-induced ovulation rate of A/J and C57BL/6J mice differed due to the action of approximately 3 to 4 loci.

The results were also examined to determine whether these estimates of number of loci were highly dependent upon extreme outlying individuals. Even if data within each strain or cross were Winzorized to replace the most extreme outliers with the second most extreme outliers, A/J and C57BL/6J differed due to the action of 5.2 loci. Since estimates of number of loci differing between strains are highly dependent on the differences of the variances of crosses, the estimate obtained after Winzorization violates the assumptions of this method. Nevertheless, these data show that our estimates of number of loci controlling induced ovulation rate are not highly dependent on the extreme individuals.

Experiment 4: number of loci by which A.SW/SnJ and SJL/J differ

Since some *H-2* haplotypes affect hormone-induced ovulation rate (Spearow *et al.*, 1983), I decided genetically to dissect out or reduce this potential source of genetic variation by using a congenic substrain of strain A/WySnJ, specifically A.SW/SnJ. A.SW/SnJ is identical to A/J mice except for the *H-2* region on chromosome 17, where A/J is *H-2^a* and A.SW/SnJ is *H-2^s* (Altman & Katz, 1979). Even though the *H-2* regions of A.SW/SnJ and SJL/J come from different strains, and differ at the closely linked *tla* serological marker, both of these strains are *H-2^s* (Altman & Katz, 1979). In essence, the *H-2* region of A.SW/SnJ is similar to that of SJL/J. Therefore, by crossing A.SW/SnJ and SJL/J to produce their *F*₁ and *F*₂ crosses, strain differences in hormone-induced ovulation rate could be examined which are largely independent of *H-2*.

Fig. 4. Distribution of hormone-induced ovulation rate of A.SW/SnJ and SJL/J strains, and their *F*₁ and *F*₂ crosses.

As shown in Fig. 4, the mean $\pm$ s.d. hormone-induced ovulation rate was 10.9 ± 5.6 ($N = 23$) for A.SW/SnJ, 47.0 ± 11.9 ($N = 24$) for SJL/J, 23.2 ± 8.0 ($N = 29$) for A.SW/SnJ $\times$ SJL/J *F*₁, and 21.9 ± 11.5 ($N = 127$) for A.SW/SnJ $\times$ SJL/J *F*₂. After square-root transformation, the mean $\pm$ s.d. hormone-induced ovulation rate was 3.191 ± 0.874 for A.SW/SnJ, 6.799 ± 0.873 for SJL/J, 4.744 ± 0.833 for A.SW/SnJ $\times$ SJL/J *F*₁ and 4.523 ± 1.203 for A.SW/SnJ $\times$ SJL/J *F*₂. The A.SW/SnJ $\times$ SJL/J *F*₁ cross showed 20% negative heterosis on a linear scale ($P < 0.01$), but no heterosis after square-root transformation. The variances of parentals and the A.SW/SnJ $\times$ SJL/J *F*₁ for square-root transformed induced ovulation rate were very similar, differing by less than 10%. In contrast, the variance of square-root transformed hormone-induced ovulation rate for the A.SW/SnJ $\times$ SJL/J *F*₂ was twice that of either parent or the *F*₁ cross. An analysis of untransformed induced ovulation rate resulted in estimates of 2.39 loci (using *F*₁ variance) and 2.88 loci (using the pooled variance) by which A.SW/SnJ and SJL/J differ. An analysis of square-root transformed data revealed estimates of 2.16 (using *F*₁ variance) and 2.26 (using the pooled variance) loci by which A.SW/SnJ and SJL/J differ with respect to hormone-induced ovulation rate. Inclusion of the effect of

Fig. 5. Hormone-induced ovulation rate of mice of the A/J and C57BL/6J strains, C57BL/6J × A/J F_1 cross, and recombinant inbred mice produced from the A/J and C57BL/6J strains. Values are mean $\pm$ s.e.m. for no. of mice shown.

dominance in a model using the F_1 variance as an estimate of environmental variance resulted in estimates of 2.52 loci (raw data) and 2.18 (square-root transformed data) by which strains A.SW/SnJ and SJL/J mice differ with respect to hormone-induced ovulation rate. Thus, regardless of how these data are analysed, the results show that the induced ovulation rate of A.SW/SnJ and SJL/J mice differ due to the action of 2 or 3 loci.

Experiment 5: comparison of hormone-induced ovulation rate in recombinant inbred strains

Recombinant inbred strains of A/J and C57BL/6J were examined to test independently whether the induced ovulation rate of mice of these strains differed due to the effect of a small number of major genetic loci. Hormone-induced ovulation rate (Fig. 5) differed amongst the recombinant inbred strains ($P < 0.0001$). Of the 5 recombinant inbred strains examined, 3 had a hormone-induced ovulation rate near that of A/J, one had an induced ovulation rate near the F_1 and one near that of C57BL/6J. These results confirm that A/J and C57BL/6J mice differ by a small number of loci controlling hormone-induced ovulation rate.

Discussion

Screening the hormone-induced ovulation rate of mice of 16 inbred strains in Exp. 1 led to the discovery of a tremendous amount of genetic variation in this trait. The 5-fold genetic differences in hormone-induced ovulation rate between A/J and SJL/J mice and 6-fold genetic differences in hormone-induced ovulation rate between A/J and C57BL/6J mice are considerably larger than the genetic differences previously reported for hormone-induced ovulation rate of mice (Fowler & Edwards, 1957; Falconer *et al.*, 1961; Bradford, 1969; Land & Falconer, 1969; Wolfe, 1971; Land *et al.*, 1974; Bindon & Pennycuik, 1974; Durrant *et al.*, 1980), sheep (Bradford *et al.*, 1971) and cattle (Chupin *et al.*, 1985). Nevertheless, screening widely divergent genotypes in other mammalian species may also reveal a magnitude of genetic variation in hormone-induced ovulation rate similar to that found in the present study.

The present study demonstrated the existence of genes with large effects on ovarian function. By administering large doses of exogenous gonadotrophins, the negative feedback controls on spontaneous ovulation rate were bypassed. Measurements of spontaneous ovulation rate would not

have bypassed the negative feedback controls of serum gonadotrophins, and it is likely that much of the genetic variation in ovarian response to gonadotrophins would have escaped detection. It seems possible that the homeostatic mechanisms controlling ovulation rate may often hide genetic variation in reproductive function. I propose that (1) if complicated reproductive traits are broken down further and further into their components and (2) if feedback regulation controlling these traits is bypassed, other major genes controlling reproductive traits and their components will be identified.

The number of old eggs ovulated in Exp. 1 of the present study, or the number of fresh eggs ovulated by 5 i.u. hCG alone (Spearow, 1988), suggests that strain differences in the maturation of follicles by endogenous gonadotrophins, which can be ovulated by 5 i.u. PMSG or 5 i.u. hCG alone, is not a major contributor to genetic differences in hormone-induced ovulation rate. These results contrast with the findings of Eleftheriou *et al.* (1972), Eleftheriou & Kristal (1974), and Spearow (1980) of large strain differences in ovulation rate induced by a single injection of gonadotrophin. Other studies suggest that the genetic differences in hormone-induced ovulation rate observed in the present study were due to genetic differences in the ovarian responsiveness to large doses of exogenous gonadotrophins (Spearow, 1988).

The present study suggests that induced ovulation rate does not fit an additive model. The magnitude and direction of dominance or heterosis in hormone-induced ovulation rate depended on the specific cross involved and differed greatly amongst crosses. The heterosis in A/J $\times$ SEC/1ReJ F_1 , A/J $\times$ DBA/2J F_1 , and C57BL/6J $\times$ DBA/2J F_1 seemed actually to be due to complete dominance of one parent. The complete dominance of the DBA/2J parent was particularly striking since mice of the A/J and C57BL/6J strains had the lowest and highest hormone-induced ovulation rates, respectively.

The hormone-induced ovulation rate of the A/J $\times$ AKR/J F_1 was twice that of either parental inbred or the mid-parental average. This heterosis in hormone-induced ovulation rate could be due to one or more loci which show overdominance. Alternatively, the heterosis of the A/J $\times$ AKR/J F_1 could be due to the complementation of the other strain's limitations at loci showing dominance. If this were the case, the hormone-induced ovulation rate of A/J and AKR/J would have been low due to different genetic limitations.

The C57BL/6J $\times$ SJL/J F_1 cross also showed true negative 'heterosis' in hormone-induced ovulation rate. The induced ovulation rate of this F_1 was lower than that of either parent or the mid-parental average. The results demonstrate that several crosses showed no dominance or heterosis for induced ovulation rate. The C57BL/6J $\times$ A/J F_1 showed no heterosis in hormone-induced ovulation rate in Exp. 2 or Exp. 3. After square-root transformation neither the A/J $\times$ SJL/J F_1 nor the C57BL/6J $\times$ 129/SvJ F_1 showed heterosis in hormone-induced ovulation rate. Similarly, after square-root transformation, the A.SW/SnJ $\times$ SJL/J F_1 did not show significant heterosis for induced ovulation rate. Thus, after correction for scale effects, it seems that these F_1 crosses show no dominance or heterosis.

The present study shows non-additive gene action controlling hormone-induced ovulation rate in some crosses but not in others. Clearly there is a large pool of genetic variation in hormone-induced ovulation rate awaiting further investigation. Other investigators have mainly examined the genetics of natural or spontaneous ovulation rate. Spearow & Bradford (1983) also found a small amount and Land *et al.* (1974) found a considerable amount of non-additive gene action controlling the natural ovulation rate of inbred strains of mice and rats, respectively. In contrast, in non-inbred lines of mice, natural ovulation rate has been shown to be controlled mainly by additive gene action (Bradford & Nott, 1969; Land & Falconer, 1969; Bradford *et al.*, 1980; Spearow & Bradford, 1983).

The broad sense heritability of hormone-induced ovulation rate in the present study seems to be much higher than that reported for most other reproductive traits. Since 70–75% of the variation in hormone-induced ovulation rate in Exp. 1 was amongst strains, these results suggest that hormone-induced ovulation rate in mice has a high heritability in the broad sense. The present studies were

conducted on inbred strains of mice and their crosses. Thus, the nature of the genetic variability amongst these specific inbred strains and their crosses could differ considerably from the genetic variability within non-inbred populations in other species. A particular major gene controlling hormone-induced ovulation rate may or may not be segregating in a given non-inbred population. Checking foundation or synthetic populations to confirm a high frequency of favourable alleles for induced ovulation rate before initiating selection should help to ensure rapid response to selection for this trait.

It has been generally assumed that most reproductive traits were controlled by many loci with small effects; i.e. that reproductive traits are quantitatively inherited (Falconer, 1960, 1981; Rice *et al.*, 1970). For example, Eklund & Bradford (1977) estimated that the 2-fold difference in litter size between a randomly selected strain of mice and a strain which had been selected for increased litter size was due to the action of between 54 and 164 loci. Nevertheless, major genes controlling reproductive traits have been detected. A major gene controls the ability of the male to induce ovulation in PMSG-primed mice (Eleftheriou *et al.*, 1972). A major gene also controls bell or flashing-light facilitation of PMSG-induced ovulation in mice (Eleftheriou & Kristal, 1974). The discovery that major genetic differences in the ovulation rate and litter size of sheep are due to the action of a single locus, the Booroola fertility locus (Piper & Bindon, 1985), has attracted further interest in other genes having major effects on reproductive performance. The present study shows that major genes controlling reproductive performance are more common and more important than was previously thought.

The present study found that a small number of major genes control very large differences in hormone-induced ovulation rate in mice. The comparison of the parental strains, F_1 and F_2 crosses revealed that the 6-fold differences in hormone-induced ovulation rate between A/J and C57BL/6J mice were due to the action of about 3 or 4 loci. These estimates are an approximation of the true number of loci differing between strains. Nevertheless, the recombinant inbred data clearly confirm that the genetic differences in hormone-induced ovulation rate between A/J and C57BL/6J are due to the action of a small number of loci. Spearow *et al.* (1983) have shown that one of these loci controlling hormone-induced ovulation rate for which A/J and C57BL/6J differ is located on chromosome 17 near the major histocompatibility locus (H-2). H-2 congenic and recombinant inbred strains of A/J and C57BL/6J provide the genetic tools needed for dissecting out the biochemical and physiological mechanisms which mediate the observed genetic differences in hormone-induced ovulation rate between these two strains.

The present study found that the 5-fold difference in the hormone-induced ovulation rate of A.SW/SnJ and SJL/J was due to the action of about 2–3 loci. Regardless of the statistical assumptions utilized, the estimates of the number of loci differing between these two strains ranged from 2.16 to 2.88 loci, averaging 2.43 loci. While A.SW/SnJ and SJL/J mice are both $H-2^s$, due to the different origins of this H-2 region in these strains of mice, it is not possible to eliminate fully a difference in H-2 between these two strains of mice. Other results from this laboratory (not shown) have revealed that the hormone-induced ovulation rate of A/J ($H-2^a$) mice does not differ from that of A/WySnJ ($H-2^a$) or from A.SW/SnJ ($H-2^{su}$) mice. This suggests that the 5-fold differences in hormone-induced ovulation rate between A.SW/SnJ and SJL/J mice, or between A/J and SJL/J mice, are both due to 2 or 3 loci.

The present study provides a foundation for more detailed studies on the genetic variation in the biochemical and physiological mechanisms controlling hormone-induced ovulation rate. Major 5- to 6-fold genetic differences in hormone-induced ovulation rate which are due to the action of a small number of loci are demonstrated and there is clearly a large pool of genetic variation in hormone-induced ovulation rate awaiting further investigation.

The present mouse model, along with the Booroola 'F' gene model in sheep (Bindon *et al.*, 1985; Piper & Bindon, 1985), should also provide valuable information on the physiological-genetic control of ovarian function in other species. While species may differ in the component traits which limit reproductive performance, the hormonal requirements for follicular maturation and ovulation

and the mechanisms controlling follicular function are relatively similar across many mammalian species. The genetic differences in hormone-induced ovulation rate observed in the present study are likely to be indicative of the kinds of genetic variation in ovarian function present in other mammalian species. Striking differences in the ovarian response to exogenous gonadotrophins have also been found within sheep, cattle and human populations (Bradford *et al.*, 1971; Jones, 1984). Unfortunately, well defined genetic models for examining hormone-induced ovulation rate are not available for many mammalian species. Therefore, once species differences in physiological controls are considered, information obtained about genetic differences in the control of hormone-induced ovulation rate in these strains of mice should be applicable to other species. A better understanding of the genetic variation in ovarian response to gonadotrophins could enhance the development of (1) improved hormonal treatments for obtaining the desired ovulation rate in livestock and humans and (2) improved selection criteria for breeding for increased ovulation rate in livestock species either through gene transfer procedures or conventional animal breeding techniques.

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