A SEX RATIO FACTOR IN THE HOUSE MOUSE THAT IS TRANSMITTED BY THE MALE[1]

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TO undertake a study of the sex ratio of mammals requires courage in the face of a vast literature that bears testimony to the capricious nature of the character. For ten years we have been studying a sex ratio factor that distinguishes two lines of mice; one line produces a consistent excess of males, the other a consistent excess of females. There have been a number of changes in environment over the years, some drastic. There have been four changes of diet, and the entire colony was moved in 1950 from the University of Saskatchewan to the University of Kansas. Subsequently there have been two changes of animal quarters. In spite of these disturbing influences the mice have continued to produce an excess of males from the "high line" (PHH) and an excess of females from the "low line" (PHL). Since these mice have been mated brother by sister for 30 generations, there can be no doubt of the fitness of the materials for study of the sex ratio. Experiments giving reproducible results will be described, particularly the experiments that confirm and extend the earlier observation (WEIR 1955, 1958) that the male is responsible for the sex ratio of litters sired by PHH and PHL males. Recently acquired inbred lines have been crossed with PHH and PHL males to provide additional information on the sex ratio.

OBSERVATIONS

Sex ratios of inbred and non-inbred lines

The sex ratio of the mouse at birth generally shows an excess of males although significant deviations from equality of sexes may be in either direction. Sex ratios at birth for six standard inbred strains are shown in Table 1. The figures, which may be taken as fairly representative, do not differ significantly from those of HOWARD, MCLAREN, MICHIE and SANDER (1955) for the three strains available for comparison: A, C57BL and DBA (substrains not designated.) Our mice were fed Purina Laboratory Chow and, more recently, Purina Mouse Breeder Chow; their mice were fed Purina Fox Chow supplemented with bread and milk and either lettuce or cabbage. Our data were collected between 1958 and 1960, theirs between 1941 and 1945. The striking agreement in sex ratios, despite larger litters from their mice, is not to be taken as *prima*

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TABLE 1

Sex ratios at birth of inbred strains A/He, AKR, C3H, BALB/C, C57BL/6 and DBA/2 and sex ratios at weaning of noninbred T, TH, and TL and inbred PHH, PHL, LCH, and LCL

Mouse strain	No. of litters	Males total	Percent males	Standard error	Mean litter size
A/He	426	973/1,904	51.1	1.14	4.5
AKR	330	968/1,905	50.8	1.14	5.8
C3H	438	1,362/2,611	52.2	0.98	6.0
BALB/C	286	680/1,296	52.5	1.39	4.5
C57BL/6	2,511	8,233/15,733	52.3	0.20	6.3
DBA/2	417	1,052/2,032	51.8	1.11	4.9
	4,408	13,268/25,481	52.1	0.003	5.8
T	456	1,884/3,796	49.6	0.81	8.3
TH	75	268/595	45.0	2.05	7.9
TL	80	349/660	52.9	1.95	8.2
PHH	388	1,310/2,479	52.8	1.00	6.4
PHL	510	1,214/2,907	41.8	0.93	5.7
LCH	307	1,114/2,245	49.6	1.06	7.3
LCL	325	1,025/2,125	48.2	1.08	6.5
A*	40	110/208	52.9	3.5	5.2
C57BL*	39	144/272	52.9	3.0	7.0
DBA*	40	127/245	51.8	3.2	6.1

* HOWARD *et al.* 1955.

facie evidence for stability of the character. On the other hand, there is no call to invoke the usual arguments concerning possible effects of environment on mortality of one or the other sex.

Litters from the pure strains A/He, AKR, C3H, BALB/C, C57BL/6 and DBA/2 were sexed at birth; some mice from the larger litters were then destroyed. Since infanticide was not random for sex, reliable sex ratios at weaning for these strains are not available. For all other strains and crosses, final sexing was done either at weaning or at 7–10 days of age. By seven days the nipples are usually conspicuous in females. Data for PHH, PHL, TH, TL, T, LCH and LCL (Table 1) are from classification at weaning.

Each of the six standard inbred strains shows an excess of males, but the departure from equality is significant only for C3H and C57BL/6. If data on weaning were available for these strains the ratios might be reduced slightly because postnatal mortality usually falls more heavily on the male. On occasion, postnatal mortality has eliminated an appreciable number of males (see discussion of Table 2).

An examination of records that include data on sex ratio, both at birth and at weaning, reveals that errors are more frequent than had been estimated by laboratory personnel. For example, in a series of 130 litters (data of Table 2), ten litters were known to be misclassified: there were nine females classed as males and four males recorded as females. From earlier work, an exact measure

of error was obtained by classifying all newborn mice, including stillbirths, first by inspection and then by dissection (WEIR, HAUBENSTOCK and BECK 1958). The investigators had the benefit of an immediate check which should have led to improvement; however, knowledge that the observations based on external morphology were not critical probably had the opposite effect. If one had to rely entirely on classification at birth one might do better, but some errors will persist under any conditions. Inspection of external features failed to make a correct separation of the sexes in 33 out of 195 litters (44 mice). The error was equally distributed between the sexes. Totals for the 33 discrepant litters were 144 females to 120 males "by inspection" and 146 females to 118 males "by dissection". From these data alone we must agree with HOWARD *et al.* (1955) that: "The only consequence to be expected from misclassification is a blurring of effects of genotype or enviroment which would otherwise stand out in sharper relief". We favor sexing at ten days or older if, over a period of time, the principal investigators cannot inspect all of the mice.

TABLE 2a

Tertiary sex ratios of F_1 litters sired by PHH and PHL males

	F_1 litters from females of inbred strains listed at left								
	× PHH males				× PHL males				
Strain of female	No. of litters	Males total	Percent males	Mean litter size	No. of litters	Males total	Percent males	Mean litter size	χ^2 (sex ratio)
PHH	..	.....	(52.8)	(6.4)	79	211/544	38.8	6.9	...
PHL	80	286/515	55.5	6.4	..	.....	(41.8)	(5.7)	...
A/He	15	60/99	60.6	6.6	29	86/227	37.9	7.8	14.4
AKR	10	39/65	60.0	6.5	13	30/88	34.1	6.8	10.1
C3H	19	51/105	48.6	5.5	24	72/169	42.6	7.0	0.9
BALB/C	10	41/75	54.7	7.5	10	32/79	40.5	7.9	3.1
	54	191/344	55.5	6.4	76	220/563	39.1	7.4	23.1

TABLE 2b

Secondary sex ratios of F_1 litters sired by PHH and PHL males

	F_1 litters from females of inbred strains listed at left								
	× PHH males				× PHL males				
Strain of female	No. of litters	Males total	Percent males	Mean litter size	No. of litters	Males total	Percent males	Mean litter size	χ^2 (sex ratio)
A/He	15	62/102	60.8	6.8	29	92/242	38.0	8.3	15.0
AKR	15	63/105	60.0	7.0	15	46/117	39.3	7.8	9.5
C3H	27	90/167	53.9	6.2	29	97/228	42.5	7.9	5.0
BALB/C	10	47/83	56.6	8.3	10	32/79	40.5	7.9	4.2
	67	262/457	57.3	6.8	83	267/666	40.1	8.0	32.3

Sex ratios for the six standard inbred strains exceed 50 percent males in each instance, but only in C3H and C57BL/6 is the excess of males significant by χ^2 test. The C3H and C57BL/6 mice produced larger litters than the other strains, a fact which might incline the casual observer to draw the inference that in general larger litters are associated with higher sex ratios. The temptation to generalize would have been stronger if the AKR strain were not represented.

The outbred T line derived from the MACARTHUR (1944) stock is of interest for several reasons: (1) The sexes are represented in equal proportions with no evidence for effective genetic variance of sex ratio as determined by FALCONER's (1954) application of ROBERTSON's (1951) *simplified maximum likelihood method*. The heterogeneity variance in sex ratio is less than its standard error; also there is no significant relationship between sex ratio and litter size. An attempt to select for the sex ratio was ineffective, as might be expected. (2) Selection for *p*H of venous blood resulted directly in production of the high and low sex ratio lines, PHH and PHL. (3) The selection experiment was repeated by WOLFE (1960), using arterial rather than venous blood and avoiding inbreeding. Selection produced high and low sex ratio lines TH and TL, but with relationships to blood-*p*H opposite from PHH and PHL.

The TH and TL lines are currently being inbred and have been subjected to only a few tests. In crosses there is not the male effect on sex ratio, as in PHH and PHL; in fact the sex ratio has tended to follow the female (WOLFE 1960). The male excess in TL is not significant with present numbers, but the difference in sex ratio between TH and TL is highly significant. The biological significance, if any, of litter size and sex ratio relations (see TH, TL, PHH, PHL in Table 1) remains unknown. A comparison of the arterio-venous differences by WOLFE (1959, 1960) shows that selections based on arterial readings had the effect of increasing the arterio-venous difference in the high line and decreasing it in the low line. The opposite is true for comparisons of the PHH and PHL lines selected on the basis of venous readings. Although the four strains differ in a number of respects, a unifying hypothesis may eventually be found. In the meantime, the experimental approach to the sex ratio problem is confined mostly to the PHH and PHL inbred strains.

The correlated response of shifts in sex ratio in PHH and PHL mice was not discovered until the data from ten generations of mice were analyzed (WEIR 1953). Not only was there no conscious selection for sex ratio, but the mating plan (using full-sib matings) would have the effect of selecting against extremes of sex ratio. Litters having a nearly equal representation of males and females contributed most of the matings, whereas mice from unisexual litters were not used to perpetuate the lines. Sex ratios and venous blood-*p*H values after one generation of selection were: PHH, 50.5 ± 3.5 percent males, *p*H 7.466 ± .0048; PHL, 41.2 ± 3.8 percent males, *p*H 7.420 ± .0051 (WEIR 1953). Selection was discontinued after three generations, and the mice have been maintained by full-sib matings. At generations 17–24, WOLFE (1959) tested the mice for blood-*p*H with the following results for venous blood: PHH 7.428 ± .0065, PHL 7.381 ± .0084. It will be seen that, even if the absolute *p*H values have

changed, the difference between strains has not (.047 from WOLFE's 1959 Table 1 and .046 from WEIR's 1953 Table 4). The same seems to be true for the sex ratio; the difference between strains remains stable even though the actual ratios have varied. Although selection for *p*H of venous blood resulted in an alteration of sex ratio, intensive efforts to establish cause and effect relations have given negative results for the most part.

The PHH and PHL strains differ in a number of respects. The greater activity of PHL mice, noted by several observers, has been measured by use of activity wheels. Eighty-eight preconditioned PHL mice turned over a mean of 1908 ± 216 revolutions in a 24-hour test period whereas 66 PHH mice, under comparable conditions, were turning over a mean of 1324 ± 175 revolutions. In addition we have found more active thyroid glands and higher levels of blood lactic acid in PHL than PHH mice; and so, investigation of the physiological bases for the *p*H differences is under way. Variations of blood-*p*H and of sex ratio may have common causes, but until something is known of the real nature of those variations no support can be found for MCWHIRTER's (1956) interesting speculations.

The LCH and LCL strains were derived from T by selection for total leucocyte count and have been inbred for more than 30 generations. The LCH strain has a mean leucocyte count of 11,000 cells/mm^3; LCL, a mean of 3700 cells/mm^3. The strains differ in a number of respects including body size and litter size. The sex ratios of LCH and LCL do not differ significantly from one another, from T or from equality. Since inbreeding *per se* did not change the sex ratio of LCH and LCL and, as already noted, the difference in sex ratio between PHH and PHL following one generation of selection was not affected by subsequent inbreeding, it seems that inbreeding depression has little to do with the character.

Sex ratios of F_1 litters

Results from reciprocal crosses between PHH and PHL have been presented in detail (WEIR 1955), and the earlier data, combined and augmented by new material, are shown in the upper portion of Table 2a. To facilitate comparisons, sex ratios and litter sizes from Table 1 for PHH and PHL are shown in parentheses.

It will be noted that the pooled data exaggerate differences in the sex ratio in reciprocal crosses as compared to *inter se* matings, but this effect is neither statistically significant nor repeatable; it is confined to two of the four replications from which the data are compiled.

Turning to litter sizes, we see that there is an increase in mean number of young from interstrain as contrasted to intrastrain matings. This observation, attributable to greater prenatal viability of hybrids than of inbreds, needs no comment. However, the dependence of sex ratio on the genotype of the sire and the seeming lack of dependence on genotype of dam or zygote requires further study. Reliance only on data from *inter se* and reciprocal cross matings does not come to grips with the proposition that there may be subtle interactions. It would be just as unwise to view the female as nothing more than an incubator for

zygotes as to assign the male only the role of initiating the processes of embryonic development. An obvious experiment is to cross PHH and PHL males to females of different inbred strains. This has now been done.

The mating unit consisted of a single male of the PHH or PHL strain and four females (A/He, AKR, C3H, and BALB/C). Ten such matings to PHH males and ten to PHL males were made up initially, but one PHH and one PHL male proved to be sterile so the 80 females were distributed among 18 mating cages. Pregnant females were isolated and checked each morning. Sex of living young was classified "at birth" (usually within 12 hours of parturition) and at 7–10 days. The figures in Table 2a are from the second classification. Litters were destroyed and the females returned to the mating cages. Most females produced two litters, some as many as four, and by the time the experiment was terminated, production had nearly ceased.

The results are clear-cut. The sex ratio of the litters (Table 2a) resembles the sex ratio of the inbred strain of the sire. The one seeming exception, C3H females × PHH males, is the result of postnatal loss of males. At birth (Table 2b) the difference between PHH and PHL sired groups from C3H females is significant. Litter size at birth for this group was scarcely larger than the litter size of inbred C3H so there may also have been differential prenatal or natal losses. However, even if a loss of males were to occur again, this would be no more than an interesting fact, unrelated to the central problem. (The sex ratios at birth and weaning for this cross are not significantly different, with unadjusted $\chi^2_1 = 0.73$.)

It might be argued that the sex ratio at birth is always the better one to use since it is generally based on larger numbers and is not distorted by postnatal losses. It should be pointed out, however, that the data of Table 2b have been corrected for initial errors of classification, and there is the added possibility that a few of the mice that died had also been misclassified. As most of our data do not contain records for sex at birth it seems best to use "weaning" or the 7–10 day figures as the standard point of reference.

Although there were too few litters at 7–10 days from BALB/C females to establish a satisfactory confidence level for the intersire difference between these particular groups, it may be noted that the sex ratios are not inconsistent with the pooled data, and there is no significant heterogeneity considering all groups.

The increase in litter size of each F_1 above mean litter sizes of the inbred strains has already been noted. Attention is now drawn to the relationship between the strain of the sire and the size and number of litters, taking into consideration that all males should have had essentially the same opportunities to mate. In nearly every instance the PHL males sired more and larger litters than PHH. The greater "efficiency" of PHL males, which will be discussed in a later section, is influenced here by the mating plan and is difficult to interpret. For first litters only, the records at birth were: A/He × PHH ♂, number of litters 6, mean litter size 6.7; A/He × PHL ♂, 10 and 7.8; AKR × PHH ♂, 9 and 7.7; AKR × PHL ♂, 10 and 6.4; C3H × PHH ♂, 12 and 6.5; C3H × PHL ♂, 10 and 8.5; BALB/C × PHH ♂, 5 and 8.2; BALB/C × PHL ♂, 5 and 8.2. Inbred PHH mice

inter se produce larger litters than PHL, but the over all fertility of PHL is higher because their reproductive span is longer (WEIR 1955). In the data from reciprocal crosses (Table 2a) the male effect on litter size is confounded with female fertility and is, therefore, unanalyzable.

This section of the paper may be summarized by the statement that the effect of the PHH or PHL male on the sex ratio of the litter is independent of the genotype of the female. The properties of the male gametes can only be assayed by introducing sperm into the female genital tract and producing viable young: therefore, it cannot be determined at present whether non random union of gametes occurs or whether X- and Y-bearing sperm are already disproportionate by the time they leave the male genital tract. In either case the genetic effect would be due to some property of the sperm and so, from a practical standpoint, the female is exonerated.

Double Matings

The technique for obtaining litters of mixed paternity from natural matings consists of placing in the mating cages males from different strains but with genetic color markers that permit detection of paternity. It must be acknowledged at the outset that KING (1918) was successful with rats in producing high and low sex ratio lines and also, but in a different connection, in obtaining litters of mixed paternity (KING 1929). There was no reason for her to combine materials and technique in one experiment, as we have done, because it was shown from outcrosses that the female parent was chiefly responsible for the sex ratio of the litter. She employed double matings to study sexual selection much as LEVINE (1958) has done recently for mice. In our case, results from reciprocal crosses and outcrosses to inbred lines as described above indicate that the sex ratio difference is wholly accountable by the influence of the sire. Mixing of sperm *in vivo*, through double matings, provides additional evidence that the sex ratio of PHH and PHL mice is a function of sperm source. At this stage in the investigation it seems important to introduce a minimum of extrinsic influences. Natural matings are preferable to artificial inseminations although sex ratios of litters from artificial inseminations (WEIR 1958) were the same as those from natural matings.

It is a matter of convenience that PHH and PHL strains are color marked so that crossbred and pure line progeny are distinguishable. The PHH mice are all of genotype *a/a*, *B/B*, *ln/ln* whereas there are two family lines of PHL, *a/a*, *b/b*, *ln/ln* and a^t/a^t, *b/b*, *ln/ln*. Since the recessive mutant allele *ln*, which causes dilution of coat color, is found in all mice of both strains, it now may be dropped from the genotypic formula. To distinguish (nonagouti) black mice from (nonagouti) brown or from (nonagouti) brown and tan, we may write the formulas as follows: PHH *a/a*, *B/B*; PHL *a/a*, *b/b* and a^t/a^t, *b/b*. All of the PHL males used in the experiment, with one exception to be noted, were a^t/a^t, *b/b*; so progeny from PHH females and PHL males were recognizable by the mosaic dominant expression of a^t that gives a tan belly even though the dorsum is black like the mother. Final classification for color and sex was made when the mice

were seven to ten days old, but sex was also classified at birth. Black mice, *B/B* or *B/b*, have recognizably darker eyes at birth than *b/b* mice, but this fact (though noted) was not used in the record.

To give each male an opportunity to compete on even terms with his cage mate, newborn males of PHH and PHL were cross-fostered. It was felt that males raised together would not fight and that the data would be more meaningful if environmental variables were held constant. Actually male mice do not usually fight severely in the presence of females, provided territorial rights are not also an issue. This type of behavioral pattern should have a selective advantage in the wild state as neither maimed nor exhausted males are very effective; the character may have become fixed in the species and persisted under laboratory conditions.

Before the cross-fostered males were old enough to use, mixed matings were made up (using capital letters to designate cages) as follows: (A) PHH ♂ 22261, born 3/2/59 and PHL ♂ 22276, born 3/10/59; (B), PHL ♂ 22275, a^t/a^t, born 3/10/59, seminal vesicles removed 4/3/59, and PHL ♂ 22229, *a/a*, born 3/3/59; (C) PHH ♂ 22260, born 3/2/59, and PHL ♂ 22247, born 3/7/59, replaced on 5/4/59 by PHL ♂ 22291, born 3/17/59. Females were first put in the cages on 4/29/59.

The mating with two PHL males, one with seminal vesicles removed, will be considered briefly as it represents a special case. Removal of the seminal vesicles prevents formation of the vaginal plug. Thirteen females were isolated following discovery of vaginal plugs, and two of them produced litters. There were eight litters produced from females with no vaginal plugs identified. The females were PHH and PHL (*a/a*) so paternity could be determined in each instance. Male 22229, non-tan, sired nine of the ten litters, 37 females and 23 males. The male without seminal vesicles, 22275, sired only one litter (six females and three males), but the mother of the litter had a vaginal plug, indicating that she had also copulated with 22229. Only ten litters were produced from mating (B) whereas matings (A) and (C) produced 24 and 18 litters, respectively. The smaller number of litters from mating (B) may be the result of sterile matings, but this lacks proof. This experimental aproach requires further work before anything but tentative conclusions can be drawn. It should be noted that the male that had not undergone surgery was the older one, and this would be reason enough for his greater success. There were no mixed litters. The results of this preliminary test indicate that, whereas production of a mixed litter is proof that both males copulated, production of a litter by only one male is not proof that the other male did not also copulate. However, the presence of a plug is usually an effective barrier to intromission. Additional tests are needed, including use of two males with seminal vesicles removed and combinations using vasectomized males.

Not much was expected from the preliminary experiment (cages A and C) which was not carefully designed (Table 3). The PHH males were older than the PHL males, and PHL male 22247 in cage (C) was in poor condition. He sired no litters and was replaced by PHL 22291. Also, the females used were all PHL

TABLE 3

Sex ratios of litters from "single" and "double" matings using pairs of PHH and PHL mice

Mating cage	Single matings ×PHH males Litters	Males total	Percent males	Mean litter size	Double matings ×PHH males Litters	Males total	Percent males	×PHL males Males total	Percent males	Mean litter size	Single matings ×PHL males Litters	Males total	Percent males	Mean litter size
a. Preliminary experiment (males of different ages)														
A	15	42/91	46.2	6.1	3	4/9	44.4	7/15	46.7	8.0	6	11/30	36.7	5.0
C	10	41/71	57.7	7.1	2	3/7	42.8	2/7	28.6	7.0	6	10/38	26.3	6.3
	25	83/162	51.2	6.5	5	7/16	43.8	9/22	40.9	7.6	12	21/68	30.9	5.7
b. Main experiment (males raised together)														
P	7	16/39	41.0	5.6	2	2/7	28.6	2/7	28.6	7.0	14	41/104	39.4	7.4
R	17	53/104	51.0	6.1	3	5/7	71.4	2/7	28.6	4.7	15	44/99	44.4	6.6
S	8	34/62	54.8	7.8	5	10/22	45.4	6/14	42.8	7.2	27	65/165	39.4	6.1
T	14	56/92	60.9	6.6	4	5/11	45.4	7/19	36.8	7.5	11	29/65	44.6	5.9
V	3	11/26	42.3	8.7	2	4/9	44.4	1/6	16.7	7.5	18	36/111	32.4	6.2
	49	170/323	52.6	6.6	16	26/56	46.4	18/53	34.0	6.8	85	215/544	39.5	6.4
c. Preliminary and main experiment combined														
	74	253/485	52.2	6.6	21	33/72	45.8	27/75	36.0	7.0	97	236/612	38.6	6.3

Percent double matings: Preliminary experiment 11.9, main experiment 10.7.

since PHH females were not available. The PHH males sired larger litters, 6.5 compared to 5.7, and twice as many litters, 25 compared to 12, as PHL males. These results are accountable by the dominance of the older males on the one hand and by the greater viability of hybrids as compared to inbreds on the other. However, there were proportionately just as many litters of mixed paternity as in the main experiment, and for this reason the data have been added to the main series. It seems that under a variety of conditions 10–12 percent of the litters will be from double inseminations, as LEVINE (1958) and GOWEN and SCHOTT (1933) obtained similar figures.

In the main series, the familiar difference in sex ratio between PHH and PHL sired mice showed up in mixed litters, but the difference (combining both experiments) is not significant ($\chi^2_1 = 1.47$). The sex ratio of mice sired by PHH males, 45.8 percent males considering all mixed litters, is not significantly different from the ratio, 52.2 percent males for PHH sired single litters. The 147 mice in 21 mixed litters are too few to permit drawing of definitive conclusions, but the indications are that (a) the sex ratio follows the male as in single litters or from artificial insemination (WEIR 1958), and (b) reduction in number of males by PHH sires may be real and bears further investigation.

Considering single litters only, several general observations may be made concerning particularly the data of the main experiment. The performance of PHH and PHL males raised together from birth should be a true indication of genetic differences in performance. There were 85 litters by PHL sires compared to 49 litters from PHH sires. The only factor that might introduce bias is the disproportionate representation of the two kinds of females. The actual matings, with expected values in parentheses, were as follows:

	PHH ♂♂	PHL ♂♂	
PHH females	11 (15)	30 (26)	41
PHL females	38 (34)	55 (59)	93
	49	85	134 $\chi^2 = 2.42$

Since preferential mating, if any, was for opposites, the inclusion of more PHH females should increase the disparity between males if it has any effect at all. There can be little doubt that PHL males are more successful than PHH regardless of whether the males are in active and fair competition (Table 3) or are isolated (Table 2). Only with a handicap to PHL, as in the preliminary experiment (Table 3), do PHH males come off best.

The frequency of mixed litters is not influenced by strain of dam. The 43 litters from PHH females included two mixed litters; the 107 litters from PHL, 14 mixed litters ($\chi^2_1 = 2.3$).

In single sire litters from double matings there was a relationship between sex ratio and strain of female; this is contrary to all previous experience. An arrangement of the data is shown in Table 4.

The data were further broken down in an attempt to isolate effects of parity, age of dam and litter size. Litter size may have more effect than parity as litters subsequent to the first were equal to or larger than first litters in PHL and

TABLE 4

Sex ratio related to strain of female
(Main series of Table 3, excluding litters of mixed paternity)

Type of mating	Number of litters	Males total	Percent males	Mean litter size
PHL♀ × PHH♂	38	140/248	56.4	6.5
PHL♀ × PHL♂	55	123/320	38.4	5.8
	93	263/568		6.1
PHH♀ × PHH♂	11	30/75	40.0	6.8
PHH♀ × PHL♂	30	92/224	41.1	7.5
	41	122/299		7.3

smaller than first litters in PHH, whereas sex ratio rose with litter size in each instance. The numbers are too small for hierarchical analysis, and to stress this aspect of the investigation, which leads to no clear-cut generalizations, would only add to an already confused literature on the sex ratio.

The females were examined each morning for vaginal plugs, and each definite plug was recorded. Some plugs may have been missed, but care was taken not to classify as a plug the soft white secretion which by casual inspection may be mistaken for one. Of 213 females with recorded vaginal plugs, 132 produced litters and 81 did not. There was no significant difference between PHH and PHL females. Of 102 females without recorded plugs, 38 produced litters and 64 did not. Again there was no difference between PHH and PHL females. Thirty litters from matings without recorded plugs survived long enough to be classified for sex and strain; 14 were sired by PHH males and 16 by PHL males. Altogether the PHL females had significantly more vaginal plugs (167 (157) plugs: 66 (75) no plugs) than did PHH females (46 (55) plugs: 36 (27) no plugs). Expected values are in parenthesis.

Mixed litters consisted of essentially equal numbers of young from PHH and PHL sperm. There were only two litters from PHH females, containing 12 PHH and six F_1 offspring. The remaining 19 litters from PHL females consisted of 60 F_1 and 69 PHL offspring. The data could be interpreted as a result of preferential union of sperm of males of the same strain as the female, but it is more likely that we are here confronted with but one aspect of a general reproductive superiority of the PHL male. It will be recalled that PHL males sired larger litters than PHH when A/He, AKR, C3H and BALB/C females were used in a balanced experiment (Table 2). It is highly unlikely that PHL males, compared to PHH, would show "superior combining ability" with four unrelated strains, although individual heterosis may be a factor. If equal numbers of PHH and PHL females had been presented to the males, and only one litter per female had been produced, we might have obtained useful information bearing on the problem of selective fertilization. However, the carefully designed experiments of KING (1929) and LEVINE (1958) also yielded equivocal results.

Observations of mating behavior of pairs of males were made in an attempt to determine just how double inseminations occur. It became evident that reproducible data could be obtained only by sharply delimiting the conditions of the experiment. This in turn made it unlikely that the observed behavior was the same as that occurring under ordinary laboratory conditions. For example, if a female in estrus is suddenly placed in a plastic cage with two males, and under full illumination, the conditions are different from those of the double mating experiment described above. Also, mating among mice is not a perfunctory performance.

PHH males are sexually more active than PHL and less easily rebuffed by the nonestrus female. On the other hand, PHL males usually proceed to the point of ejaculation with less prior activity than PHH males. It cannot be stated with certainty that double inseminations have been observed, as the litters produced were eaten by the mothers and not classified. It seems that a rapid round robin, finally terminated by formation of a vaginal plug by the last male to mount, is the way in which double inseminations occur. It also seems that the greater fertility of the PHL male is, in part, a function of behavioral pattern. Circumstantial evidence indicates that PHH and PHL males, isolated from one another, mate at different times in relation to the estrus cycle of the female.

Conflicting evidence concerning the relation of the sex of offspring to the time of coitus during the estrus cycle (CREW 1927, 1952) has, up to now, discouraged us from performing the appropriate experiments. The remarkable discovery of BRADEN (1958) that mating late in the estrus cycle significantly reduced the "segregation ratio" advantage of *t* alleles (*versus T*) is suggestive. But until similar tests, using PHH and PHL males, have been carried to completion there is neither reason to compare our findings with those from other materials nor to emphasize unduly the unique aspects of our materials.

SUMMARY

(1.) Results from a ten-year study of a sex ratio factor in the house mouse have been presented. PHH and PHL strains derived by selection for venous blood-*p*H and maintained by brother-sister matings for 30 generations have consistently shown a significant difference in sex ratio (PHH 52.8 ± 1.00 percent males, PHL 41.8 ± 0.93 percent males at weaning).

(2.) Secondary sex ratios of standard inbred strains under the same laboratory conditions as PHH and PHL were: A/He 51.1 ± 1.14; AKR 50.8 ± 1.14; C3H 52.2 ± 0.98; BALB/C 51.6 ± 1.39; C57BL/6 52.3 ± 0.20; DBA/2 51.8 ± 1.11. The departure from equality (male excess) is significant for C3H and C57BL/6.

(3.) The outbred T line from which PHH and PHL were derived has equality of sexes. Sex ratios of lines derived by selection for arterial blood-*p*H were: 45.0 ± 2.05 in the high blood-*p*H line (TH) and 52.9 ± 1.95 in the low line (TL). Inbred strains derived from T by selection for total leucocyte count did not differ from T in sex ratio, indicating that inbreeding alone does not alter the sex ratio.

(4.) An earlier finding that the sex ratio follows the male parent in reciprocal crosses of PHH and PHL has been extended to crosses with standard inbred strains. From each cross, using PHH and PHL males, the sex ratio of the litter resembled the sex ratio of the strain of the sire. Tertiary ratios of F_1 litters from A/He, AKR, C3H, and BALB/C dams were: by PHH males, 55.5 percent males; by PHL males, 39.1 percent males.

(5.) Twenty-one mixed litters from double inseminations contained 72 mice from PHH sperm (33 males) and 75 mice from PHL sperm (27 males).

(6.) PHL males sired significantly more litters than PHH males of like age when males were in active competition (one PHH and one PHL male per mating cage) and also when only one male per mating cage was used.

(7.) The PHL males are more often successful in copulating with females in estrus than PHH males, although PHH males are more active.

(8.) Age of dam, parity, and litter size did not influence the sex ratio in any consistent manner.

(9.) Experiments are in progress to determine the effect on the sex ratio of time of mating during the estrus cycle.

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