

## **‘Heterosis’ in litter size of chimaeric mice**

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### SUMMARY

Aggregation chimaeras were made from embryos of C57BL/6 and BALB/c mice. Chimaeric and control females were mated with ICR males at 8 weeks of age and their litter sizes were evaluated over a 5-month period after the first mating. Progeny tests showed that 18 of 27 chimaeras produced oocytes of both genotypes. The mean litter sizes of C57BL/6, BALB/c and their  $F_1$  crosses (C57BL/6  $\times$  BALB/c and BALB/c  $\times$  C57BL/6) were 8.14, 9.36, 13.38 and 13.40, respectively. The mean for chimaeras was 11.54 and chimaeric heterosis was evident, but it was not as much as heterosis in the  $F_1$ . When the chimaeras were classified into the mixed and single-genotype progeny chimaeras, chimaeric heterosis was observed only in the mixed-progeny chimaeras. Quantitative GPI analyses in ten organs showed that the degree of chimaerism in the mixed-genotype progeny chimaeras was higher than that in most of the single-genotype progeny chimaeras and that the degree of chimaerism in the ovaries was positively correlated with litter size in the mixed-genotype progeny chimaeras. On the other hand, such correlation was not observed in the single-genotype progeny chimaeras.

### 1. INTRODUCTION

Chimaeric mice are generally known to be vigorous and the possibility of ‘vegetative heterosis’ in chimaeric animals has been predicted (McLaren, 1976). Heterosis in the ordinary sense is a genetic phenomenon resulting from heterozygosity and is manifested in quantitative traits. Although heterosis has been of interest for many years, its mechanism has yet to be explained in biochemical-physiologic terms.

Aggregation chimaeric mice made from embryos derived from different inbred strains contain genetically different populations of cells, but their cells have the same genetic constitutions as one or other of the component inbred strains. If such mice showed chimaeric heterosis, chimaeric mice would provide a unique experimental system for physiological studies on heterosis. There is, however, little experimental evidence to support this phenomenon. Some evidence has been presented for body weight in aggregation chimaeric mice (Falconer *et al.* 1981), but to our knowledge, no study has examined the evidence for chimaeric heterosis for reproductive traits.

The present investigation was undertaken to examine (*a*) whether aggregation

chimaeric mice show chimaeric heterosis for litter size, and if so, (b) whether the degree of chimaerism affects the heterosis.

## 2. MATERIALS AND METHODS

Aggregation chimaeric embryos of C57BL/6CrJms (black, Gpi-1<sup>b</sup>/Gpi-1<sup>b</sup>) and BALB/cCrJms (albino, Gpi-1<sup>a</sup>/Gpi-1<sup>a</sup>) were produced by the method of Awata, Onishi & Muramatsu (1984). After removal of the zona pellucida with pronase (Minz, 1962), pairs of 8-cell embryos were placed in each well of a microtitration plate (96 wells, 8 × 12.5 cm) filled with modified Whitten's medium (Hoppe & Pitts, 1973). The plate was centrifuged at 1500–2000 rev./min for 5–10 min at room temperature to encourage the pairs to make contact. The plate was then transferred to a chamber at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95% air. Paraffin oil was not used in any of the procedures. After culture for about 20 h, chimaeric embryos were picked up from the plate and transferred to uteri of pseudopregnant foster mothers of ICR random-bred mice (albino). In our laboratory, 20–40 pairs of embryos were treated on one plate and transferred to 2–4 foster mothers.

Of the 87 mice born to 21 dams, 73 were overtly chimaeric as judged by coat colour mosaicism. None of the animals lacking coat mosaicism were found to be chimaeras by glucose phosphate isomerase (GPI) analyses. Twenty-eight overt chimaeras were female and were used in this study, but one died before mating. The two component strains and their reciprocal F<sub>1</sub> crosses were used as control animals. Litter sizes of the foster mothers producing chimaeras were small, averaging about four. The mother's body weight is negatively correlated with the size of the litter in which she was born, but positively with the size of her own litter (Falconer, 1960). Therefore, the size of each litter from which the controls were obtained was standardized to four pups, which were nursed by an ICR dam on the day of parturition to minimize maternal effects on pup growth.

The chimaeric and control females were mated at 8 weeks of age. To achieve high virility ICR was used as the sire line and pair matings were employed. All progeny were thus expected to be non-inbred, thus preventing any bias due to inbreeding depression of progeny born to chimaeric and control females. The dam was allowed to litter down in the presence of the sire and sizes of successive litters up to the 5th litter were recorded at birth over a 5-month period after the first mating.

The coat colour of the progeny was also recorded to give an indication of whether functional gametes of one or both genotypes of the component strains were being produced and the chimaeric dams were classified into mixed- and single-genotype progeny chimaeras.

The chimaeras were sacrificed at 7–8 months of age. The relative contribution of each genotype to the erythrocytes, heart, intestine, kidney, liver, lung, spleen, stomach, ovary and uterus were determined by quantitative GPI analyses. One volume of washed packed erythrocytes was mixed with five volumes of distilled water and tissue samples were homogenized in 20 volumes of distilled water. Electrophoresis was performed on Titan III Iso-vis cellulose acetate plates (Helena Laboratories), with phosphate buffer (pH 6.8,  $\mu = 0.1$ ) at 160 V for 1 h.

After electrophoresis the plate was affixed to another one that was impregnated with the staining mixture described by Eicher & Washburn (1978).

The relative intensities of the allozymes on the overlay plates were measured on a Cliniscan densitometer (Helena Laboratories). The method is simple, but densitometric results obtained from experimental mixtures containing various

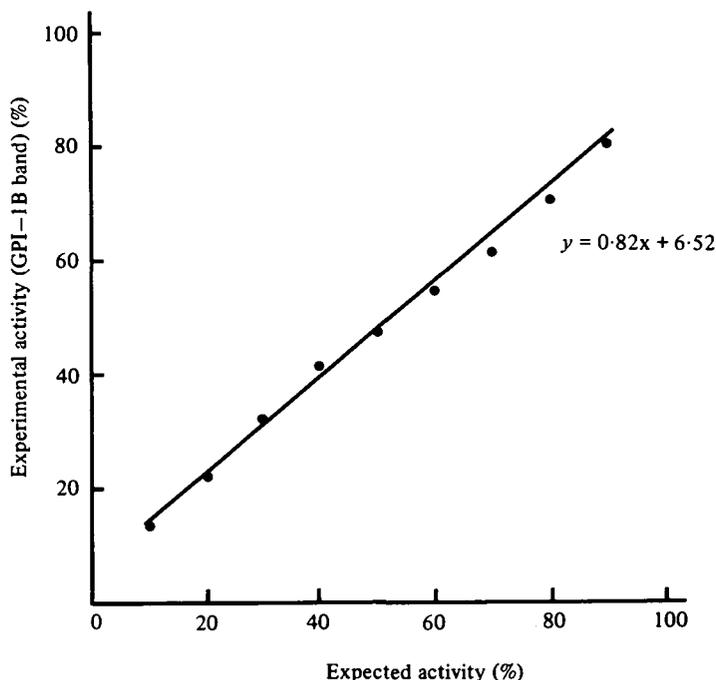


Fig. 1. Accuracy of densitometric results. Various mixtures of GPI-1A and GPI-1B homogenates of liver were prepared and electrophoresed. The observed proportion of GPI-1B enzyme was plotted against the expected proportion based on the composition of the experimental mixture.

known proportions of fast- and slow-migrating GPI did not completely agree with the expected values (Fig. 1). However, the relationship between the experimental and expected values was almost linear until the proportion 10:90 was reached. The relative staining intensities in this range were then adjusted by the regressions estimated from the experimental mixture of haemolysates or of homogenates of each organ. If the adjusted proportion of either component was over 90%, the value was considered unreliable and was therefore treated as 100%.

### 3. RESULTS

Table 1 shows the results of the GPI analyses and breeding test on female chimaeras. Chimaera three died immediately after the breeding test and GPI analyses were not carried out on this animal. Of the 27 chimaeras studied, 18 produced mixed-genotype progenies. In these chimaeras, 14 animals showed

statistically significant predominance for one progeny genotype, while the other four had almost equal frequencies.

In the mixed-genotype progeny chimaeras, the mean percentage of the BALB/c component was 38.6 in the progeny, whereas in the ovaries it was 66.7, and this

Table 1. *Analyses of chimaeras*

Chimaera no.	% BALB component in		No. of progeny (no. of litters)	% albino genotype (BALB × ICR) in progeny
	Ovary	Other organs*		
Mixed-genotype progeny chimaeras				
1	63.1	53.1	68 (5)	16.2
4	70.2	59.9	63 (5)	14.3
5	64.9	51.5	66 (5)	43.9
6	57.2	54.8	66 (5)	81.8
7	58.3	53.5	61 (5)	19.7
8	59.9	56.8	62 (5)	71.0
9	89.3	79.2	58 (5)	29.3
10	75.5	61.8	55 (5)	14.5
13	38.7	41.3	42 (3)	7.1
15	68.1	64.7	64 (5)	78.1
16	86.7	77.0	52 (5)	30.8
17	64.7	71.7	59 (5)	59.3
18	54.8	58.0	72 (5)	44.4
19	75.0	61.4	69 (5)	10.1
20	55.0	66.6	56 (4)	25.0
21	65.7	41.0	69 (5)	5.8
22	72.6	54.2	42 (4)	47.6
23	81.7	59.4	61 (5)	95.1
Mean	66.7	59.2	60.3 (4.8)	38.6
S.E.	2.9	2.6	2.0 (0.1)	6.6
Single-genotype progeny chimaeras				
2	0.0	22.3	33 (4)	0.0
3	—	—	51 (5)	0.0
11	47.8	40.4	48 (5)	0.0
12	12.1	23.0	28 (4)	0.0
14	88.2	86.6	48 (5)	100.0
24	59.6	39.6	39 (4)	0.0
25	11.3	20.0	33 (4)	0.0
26	11.1	19.7	37 (4)	0.0
27	100.0	97.7	41 (4)	100.0
Mean	41.3	43.7	39.8 (4.3)	22.2
S.E.	13.6	11.0	2.6 (0.2)	14.7

\* Mean proportion of GPI-1A in nine organs (erythrocytes, heart, intestine, kidney, liver, lung, spleen, stomach and uterus).

difference was significant ( $P < 0.001$ ). The mean of the component in the nine organs except for the ovaries was 59.2% and also significantly different from that in the progeny ( $P < 0.01$ ). The percentage of the BALB/c component in the ovaries was significantly correlated with the mean of the nine organs ( $r = 0.64$ ,  $P < 0.01$ ), but not correlated with that in the progeny ( $r = 0.10$ ).

Of the nine single-genotype progeny chimaeras, seven produced C57BL/6

genotype oocytes. The mean percentage of the BALB/c component was 41.3 in the ovaries and 43.7 in the other organs, and they were highly correlated ( $r = 0.94$ ,  $P < 0.01$ ). There was a general correspondence between the predominant genotypes in the ovaries and the genotypes of the progeny except for chimaeras 11 and 24. The mean percentage of the BALB/c component in the ovaries was 23.7 in the chimaeras which produced only C57BL/6 progenies and 94.1 in those which produced only BALB/c progenies.

Table 2. Comparison of reproductive performances of chimaeras with those of component strains and their  $F_1$  crosses when they were mated with ICR males

Dam	No. of dams		No. of litters	Litter size at birth	Percentage* of heterosis
	Mated	Littering			
Chimaera					
Mixed-progeny	18	18	86	12.62 ± 0.25†	44.2
Single-progeny	9	9	39	9.18 ± 0.45	4.9
Over-all	27	27	125	11.54 ± 0.34	31.9
$F_1$					
C57BL × BALB	25	25	123	13.38 ± 0.21	52.9
BALB × C57BL	25	25	120	13.40 ± 0.23	53.1
Over-all	50	50	243	13.39 ± 0.15	53.0
Component strain					
C57BL	25	22	87	8.14 ± 0.34	
BALB	25	23	107	9.36 ± 0.27	

\*  $[(\text{Chimaera or } F_1 - \bar{P})/\bar{P}] \times 100$ , where  $\bar{P} = (\text{C57BL} + \text{BALB})/2$ .

† Mean ± s.e.

Table 2 shows the comparison of mean litter sizes of the chimaeras with those of control animals. All females were pregnant, but two of the BALB/c and three of the C57BL/6 females died of dystocia at their first parturitions. The mean litter size of BALB/c was 1.2 larger than that of C57BL/6 and this difference was statistically significant ( $P < 0.01$ ). The  $F_1$  crosses showed a very high degree of heterosis for litter size and reciprocal crossbred differences were not observed. The over-all mean of chimaeras was 2.70 larger than the mean of C57BL/6 and BALB/c. This difference was highly significant ( $P < 0.001$ ); that is, chimaeric heterosis for litter size was evident. However, the mean litter size of chimaeras was significantly smaller than the  $F_1$  ( $P < 0.001$ ), and this indicated that chimaeric heterosis was not as much as heterosis in the  $F_1$ .

The differences between the mixed-genotype and single-genotype progeny chimaeras was 3.44 and it was highly significant ( $P < 0.001$ ). The mean of the mixed-genotype progeny chimaeras was larger than the mean of the component strains ( $P < 0.001$ ) and they showed a high degree of heterosis. In contrast, the difference between the single-genotype progeny chimaeras and the mean of the component strains was not significant and this indicated no chimaeric heterosis in the single-genotype progeny chimaeras.

Fig. 2 shows the relation between the mean litter size and the degree of

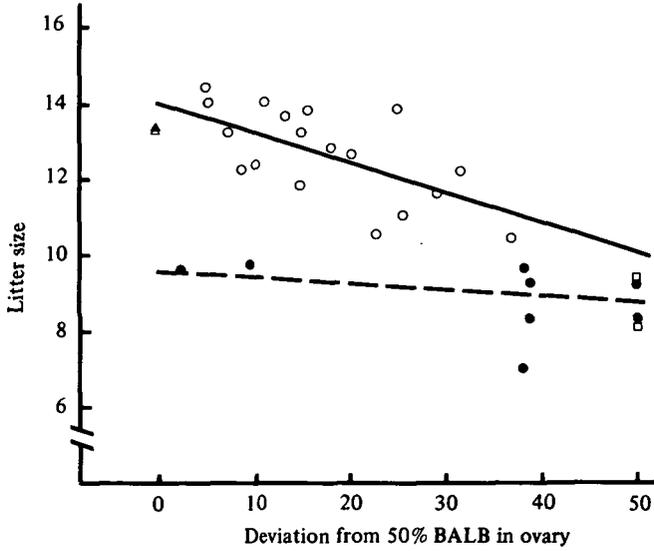


Fig. 2. Mean litter size in relation to the degree of chimaerism in ovaries. Mean litter size was plotted against the deviation from 50% of BALB/c component. ○, Mixed-genotype progeny chimaeras; ●, single-genotype progeny chimaeras; open squares, means of the inbred strains; open triangles, means of the F<sub>1</sub> crosses. —, Linear regressions in the mixed-genotype progeny chimaeras; ---, single-genotype progeny chimaeras.

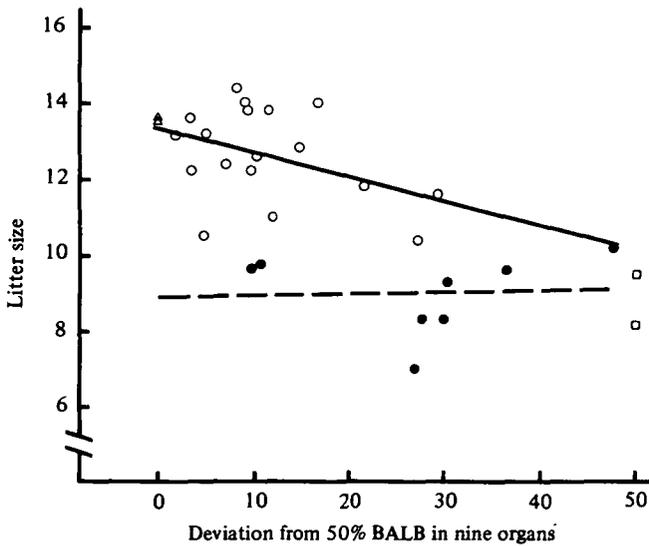


Fig. 3. Mean litter size in relation to the degree of BALB/c component in nine organs. See Fig. 2 for symbols and regressions.

chimaerism in the ovaries. The deviation from 50 % of the BALB/c component was used as a measure of the degree of chimaerism, 0 % thus indicating the highest chimaerism. The regression of litter size on the degree of chimaerism in the mixed-genotype progeny chimaeras was statistically significant ( $b = -0.076 \pm 0.023$ ,  $P < 0.01$ ). The single-genotype progeny chimaeras appeared to have a low

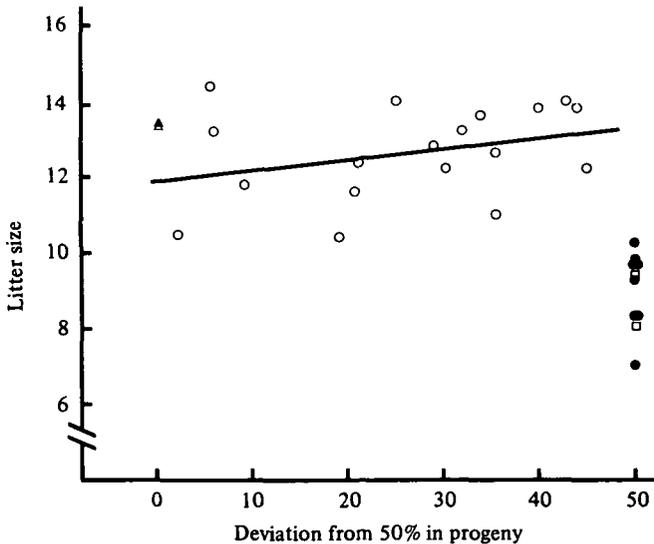


Fig. 4. Mean litter size in relation to the degree of chimaerism in germ cells of ovaries estimated from progeny genotypes. Mean litter size was plotted against the deviation from 50 % of BALB/c component in progeny. See Fig. 2 for symbols.

degree of chimaerism relative to the mixed-genotype progeny chimaeras except for two animals. These two animals, chimaeras 11 and 24, showed a high degree of chimaerism, but their litter sizes remained at a low level. The regression in the single-genotype progeny chimaeras was not significant ( $b = -0.017 \pm 0.024$ ).

Fig. 3 shows the relation between the mean litter size and the mean of chimaerism in the nine organs. The mean litter size of the mixed-genotype progeny chimaeras had a tendency to increase with increase in chimaerism in the organs, but the regression was not significant ( $b = -0.059 \pm 0.036$ ). The single-genotype progeny chimaeras showed a low degree of chimaerism except for chimaeras 11 and 24 like that in the ovaries, and the regression was not significant ( $b = 0.005 \pm 0.034$ ).

These results suggest that chimaeric heterosis depends mainly on chimaerism in the ovaries in the mixed-genotype progeny chimaeras. However, there was no heterosis in the single-genotype progeny chimaeras even if the degree of chimaerism in the ovaries was quite high. The ovaries of the mixed-genotype progeny chimaeras consist of two genetically distinct populations of somatic cells and two populations of germ cells, whereas those of the single-genotype progeny chimaeras consist of two populations of somatic cells and single population of functional germ cells. It seems likely that chimaerism in germ cells is necessary for chimaeric

heterosis in litter size. Since chimaerism in germ cells was not directly examined in this study, we estimated it from the genotypes of the progeny.

The mean litter sizes were plotted against the deviations from 50% of the BALB/c component in the progeny in Fig. 4. The regression in the mixed-genotype progeny chimaeras was clearly non-significant ( $b = 0.0258 \pm 0.0213$ ) and so this result gives no support to the idea that chimaeric heterosis depends on the degree of chimaerism in the functional germ cells.

#### 4. DISCUSSION

Falconer *et al.* (1981) suggested that chimaeric mice show 'heterosis' for body weight, but indicated that the evidence was not completely convincing. The results obtained in this study have shown that C57BL/6  $\leftrightarrow$  BALB/c females do show chimaeric heterosis for litter size, although it is not quite as much as heterosis in the F<sub>1</sub>. McLaren (1976) predicted that the presence of two genetically distinct populations of cells in one individual might confer chimaeric heterosis.

When the animals were classified into the mixed- and single-genotype progeny chimaeras by the progeny test, chimaeric heterosis was observed only in the mixed-genotype progeny chimaeras. Most single-genotype progeny chimaeras showed a low degree of chimaerism in the ten organs studied. This finding is in agreement with earlier works (Mullen & Whitten, 1971; Gearhart & Oster-Granite, 1981) and suggests that the degree of chimaerism affects the heterosis. In fact, a significant relationship was found to exist between the degrees of chimaerism in the ovaries and litter size in the mixed-genotype progeny chimaeras. Two single-genotype progeny females (chimaeras 11 and 24), however, showed small litter sizes even with a high degree of chimaerism in the ovaries.

We assumed that the presence of genetically different populations of functional germ cells in the ovaries might confer the heterosis. However, our finding that the degree of chimaerism in the germ cells estimated from the genotypes of the progeny was not correlated with litter size did not provide any evidence in support of this assumption. We did not observe wide variations in the proportions of genotypes between litters within dams. Minz (1968) pointed out that in females, where the diploid mitotic proliferative phase of gametogenesis is completed well before birth, the germ-cell population in the adult should be stable. The chimaeras were mated with ICR males and so all their progenies were crossbred. Since crossing reduces embryo mortality (McCarthy, 1965), high embryo mortality is unlikely in either of the embryo genotypes. There is thus probably no great discrepancy between the estimates made by the analysis of the breeding record and true relative proportions of the germ-cell types in the ovaries of the adult chimaeras. Minz (1969) reported that in males there was no relation between the genetic composition of the germ cells and that of the rest of the testis.

In the mixed-genotype progeny chimaeras, the predominant component was BALB/c in the ovaries and C57BL/6 in the progeny. In contrast, there was a good correspondence between the predominant genotypes in the ovaries and the genotypes of the progeny in the single-genotype progeny chimaeras except chimaeras 11 and 24. These results imply that the interaction between genetically

different somatic and germ cells, as well as the interaction between somatic cells, plays a role in the heterosis effect. Although the genetic content of germ cells is not subject to contamination by the cellular environment (McLaren, 1975), mammalian follicular oocytes are metabolically coupled to their adjacent granulosa cells via gap junctions during oocyte growth (Amsterdam *et al.* 1976; Anderson & Albertini, 1976; Gilula, Epstein & Beers, 1978; Moor, Smith & Dawson, 1980). The interaction, however, cannot explain the small litter sizes of chimaeras 11 and 24, because these females, which produced C57BL/6 oocytes, had substantial amounts of BALB/c somatic cells in the ovaries.

Another possible explanation is that the sex chromosome constitution of chimaeras 11 and 24 was different from that of the other animals. Although no quantitative assessment of reproductive abilities of XX/XY chimaeras has been made, Milet, Mukherjee & Whitten (1972) suggested that the admixture of XX and XY cells causes various degrees of abnormality in sexual development. Many XX/XY chimaeras develop as phenotypically normal fertile males (see McLaren, 1976) and some fertile XX/XY females have been examined (Ford *et al.* 1975; Evans, Ford & Lyon, 1977; Gearhart & Oster-Granite, 1981). Evans *et al.* (1977) suggested that only the XX/XY animals that are predominantly of XX cells develop into females. Gearhart & Oster-Granite (1981) reported that three of their four XX/XY females were fertile and predominantly of the female genotype, but one female, with a good 'mix' of the two genotypes, produced no progeny. If we accept this view, it is unlikely that the sex chromosome constitution of chimaeras 11 and 24, with a high degree of chimaerism, was XX/XY. Thus the reason for the small litter sizes of these two animals remains obscure.

Although a linear relationship was found to exist between litter size and the degree of chimaerism in the ovaries in the mixed-genotype progeny chimaeras, we cannot explain the difference of litter size between the mixed and single-genotype progeny chimaeras by this relationship. Clearly more data are needed in order to conclude that none of the single-genotype progeny chimaeras show the heterosis even though the degree of chimaerism in their ovaries is quite high.

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