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A maternal genetic effect on the composition of mouse aggregation chimaeras

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Summary

Two series of $12\frac{1}{2}$ day mouse chimaeric conceptuses were produced by aggregating $(C57BL \times CBA)F_2$ strain preimplantation embryos with embryos that differed at the *Gpi-1s* locus that encodes glucose phosphate isomerase, GPI-1. The composition of individual issues was evaluated by quantitative electrophoresis to estimate the % GPI-1A in the chimaeric tissue containing GPI-1A and GPI-1B. In one series of chimaeras, the GPI-1A cells were derived from a backcross between inbred BALB/c strain females and $(BC \times BALB/c)F_1$ males, where BC is the partly congenic strain C57BL/Ola.AKR-*Gpi-1s*^{a},*c*/Ws. In the other series of chimaeras, the GPI-1A cells were derived from the reciprocal backcross between $(BC \times BALB/c)F_1$ females and inbred BALB/c strain males. The [$(BC \times BALB/c)F_1$ female $\times$ BALB/c male] $\leftrightarrow$ $(C57BL \times CBA)F_2$ series of chimaeras was reasonably balanced so that GPI-1A and GPI-1B cells were fairly equally represented in the foetuses, placentas and extraembryonic membranes (tissue means: 37–51 % GPI-1A). This series did not differ significantly in composition from an earlier series of $(BC \times BALB/c)F_2 \leftrightarrow (C57BL \times CBA)F_2$ chimaeras. However, the [BALB/c female $\times$ $(BC \times BALB/c)F_1$ male] $\leftrightarrow$ $(C57BL \times CBA)F_2$ series of chimaeras was unbalanced, with mean tissue compositions (28–33 % GPI-1A) that were intermediate between the above two balanced series and the unbalanced $(BALB/c \times BALB/c) \leftrightarrow (C57BL \times CBA)F_2$ series (tissue means: 14–22 % GPI-1A), that was studied previously. Thus, both $(BALB/c \times BALB/c)$ and [BALB/c $\times$ $(BC \times BALB/c)F_1$] embryos contributed less to the tissues of chimaeric conceptuses than either $(BC \times BALB/c)F_2$ or [$(BC \times BALB/c)F_1 \times$ BALB/c] embryos. This implies that embryos from BALB/c mothers contributed less to the tissues of chimaeric conceptuses than embryos from $(BC \times BALB/c)F_1$ mothers. We, therefore, conclude that a maternal genetic effect is responsible for some of the differences in composition among the four groups of chimaeras. This maternal effect must act before the 8-cell stage but it is not yet known whether it is mediated via cytoplasmic inheritance, genomic imprinting or by the reproductive tract. Evidence that a maternal effect retards preimplantation development of embryos from BALB/c females is reviewed and the possibility that this might cause them to contribute poorly to chimaeric conceptuses when aggregated with more precociously developing embryos is discussed.

1. Introduction

In a previous study (West & Flockhart, 1994), we showed that the composition of two series of $12\frac{1}{2}$ day mouse chimaeric conceptuses differed markedly. Each series was produced by aggregating $(C57BL \times CBA)F_2$ strain preimplantation embryos (name abbreviated to BF_2 embryos) with embryos that differed at the *Gpi-1s* locus that encodes glucose phosphate isomerase, GPI-1. In one series of chimaeras, the GPI-1A cells were derived from inbred BALB/c strain embryos and in the other series the GPI-1A cells were derived from $(BC \times BALB/c)F_2$ embryos (name abbreviated to AF_2 embryos), where BC is the partly congenic strain C57BL/Ola.AKR-*Gpi-1s*^{a},*c*/Ws. (The details and abbreviated names of the relevant stocks of mice are shown in Table 1.)

The series of BALB/c $\leftrightarrow$ BF_2 chimaeras was clearly unbalanced, so that the cells derived from the BALB/c embryos were under-represented in the foetuses, placentas and extraembryonic membranes (with tissue

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Table 1. *Genotypes of the stocks of mice used to produce chimaeric conceptuses*

Abbreviated stock name	Details	Genotype	
		Albino	*Gpi-1s*
C57BL	C57BL/OlaWs	*C/C*	*b/b*
CBA	CBA/Ca	*C/C*	*b/b*
BALB/c	BALB/c/Eumm	*c/c*	*a/a*
BC	C57BL/Ola.AKR-*Gpi-1s*^{a},*c*/Ws	*c/c*	*a/a*
CC	C57BL-*Gpi-1s*^{c},*c*/Ws	*c/c*	*c/c*
CALB	BALB/c-*Gpi-ls*^{c}/Ws	*c/c*	*c/c*
AF_1	(BC female × BALB/c male)F_1 hybrid	*c/c*	*a/a*
AF_2	(AF_1 female × AF_1 male)F_2 hybrid	*c/c*	*a/a*
BF_1	(C57BL/Ola female × CBA/Ca male)F_1 hybrid	*C/C*	*b/b*
BF_2	(BF_1 female × BF_1 male)F_2 hybrid	*C/C*	*b/b*
CF_1	(CC female × CALB male)F_1 hybrid	*c/c*	*c/c*

means of 14–22% GPI-1A). In contrast, the composition of a series of $AF_2 \leftrightarrow BF_2$ chimaeras was more balanced (tissue means: 41–50% GPI-1A). The uniformly unbalanced phenotype of the BALB/c ↔ BF_2 series could be a result of (1) reduced viability of chimaeras with a high proportion of BALB/c cells, (2) generalized cell selection against BALB/c cells or (3) preferential allocation of most of the BALB/c cells in the blastocyst to the mural trophectoderm, which has a limited mitotic potential and so contributes little to the mid-gestation conceptus.

In order to investigate further the genetic basis for the genotypic imbalance we have now produced two further series of chimaeras. As before, the genetically pigmented (*C/C*), *Gpi-1s*^{b}/*Gpi-1s*^{b} component was derived from BF_2 embryos. The genetically albino (*c/c*), *Gpi-1s*^{a}/*Gpi-1s*^{a} component was derived from reciprocal backcrosses between inbred BALB/c mice and (BC × BALB/c)F_1 hybrids (designated AF_1 hybrid mice; Table 1).

The embryos derived from the reciprocal backcrosses in the two new series of chimaeras [(AF_1 female × BALB/c male) ↔ BF_2 and (BALB/c female × AF_1 male) ↔ BF_2] differed with respect to the sex of their inbred BALB/c parent but should have had a similar range of genetic diversity (with an average of 75% BALB/c genotype). Also, the males from each cross would have had the BALB/c Y chromosome because AF_1 males had BALB/c fathers. The four series of chimaeras were transferred to the same uterine environment (a third F_1 hybrid mouse strain) to avoid maternal influences on the postimplantation stages of development.

If the poor contribution of BALB/c embryos was entirely a result of genetic differences between BALB/c, BF_2 and AF_2 embryos and no parental effects were involved, then (AF_1 female × BALB/c male) backcross embryos should make similar contributions to reciprocal (BALB/c female × AF_1 male) backcross embryos. If the maternal genotype was influential, (AF_1 female × BALB/c male) embryos would be expected to make a significantly greater contribution than (BALB/c female × AF_1 male) embryos. Conversely, if the paternal genotype was influential, (BALB/c female × AF_1 male) embryos would be expected to make a significantly greater contribution than (AF_1 female × BALB/c male) embryos. The results described below imply that a maternal genetic effect is involved.

2. Materials and methods

(i) *Production of chimaeras*

Three groups of F_1 mice were produced from the stocks shown in Table 1. (The female parent is shown first in all crosses.) CBA/Ca males were obtained from the Institute of Cell, Animal and Population Biology, University of Edinburgh, BALB/c/Eumm and some BF_1 mice were purchased from the Department of Medical Microbiology, University of Edinburgh. All other animals were bred and maintained under conventional conditions in the Centre for Reproductive Biology.

BALB/c, AF_1 and BF_1 females were superovulated by injecting 5 IU pregnant mares' serum gonadotrophin (PMSG) at approximately 12.00 h followed 48 h later by 5 IU human chorionic gonadotrophin (hCG). Females were housed with males from the appropriate strain and mating was verified the following morning by the presence of a vaginal plug; the day of the vaginal plug was designated $\frac{1}{2}$ day *post coitum* (*p.c.*). Embryos produced in this way were (AF_1 female × BALB/c male) backcross, (BALB/c × AF_1) backcross and ($BF_1 \times BF_1$)F_2 (abbreviated to BF_2). On the day that the vaginal plugs were found, a group of CF_1 females was examined and those in oestrus were mated to vasectomized CF_1 males to provide homozygous *Gpi-1s*^{c}/*Gpi-1s*^{c} pseudopregnant females.

Preimplantation embryos were flushed from the reproductive tract of pregnant BALB/c, AF_1 and BF_1

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Table 2. *Strain combinations of the four series of chimaeras*

Chimaera group	Embryos aggregated* Embryo 'A'	↔	Embryo 'B'	Strain of pseudopregnant recipient female
XM†	$AF_1 \times AF_1$	↔	$BF_1 \times BF_1$	CF_1
XP	$AF_1 \times BALB/c$	↔	$BF_1 \times BF_1$	CF_1
XN	$BALB/c \times AF_1$	↔	$BF_1 \times BF_1$	CF_1
XR†	$BALB/c \times BALB/c$	↔	$BF_1 \times BF_1$	CF_1

* See Table 1 for full explanation of strain designations.
† See West & Flockhart (1994).

females at $2\frac{1}{2}$ days *p.c.* (usually between 09.00 and 11.00 h) and aggregated to produce chimaeras as previously described (West & Flockhart, 1994). The following day, the aggregated embryos were transferred to the uterus of a CF_1 pseudopregnant female. Pseudopregnant females were anaesthetized with 0·25 ml per 30 g body weight of a 1:1 v/v mixture of a 50% aqueous dilution of Hypnorm (0·315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen Pharmaceuticals) and a 50% aqueous dilution of Hypnovel (2 mg/ml midazolam hydrochloride; Roche). (In our previous description, we failed to mention the 50% dilution of the stocks of Hypnorm and Hypnovel; West & Flockhart, 1994.) Pregnancies were timed according to the pseudopregnant female.

Two groups of chimaeras (series XP and XN) were produced and compared to our previous series XM and XR. See Table 2 for details of strain combinations.

(ii) *Analysis of chimaeras*

Females were killed at $12\frac{1}{2}$ days gestation and the conceptuses were dissected as described by West & Flockhart (1994) to provide foetus, amnion, visceral yolk sac mesoderm, visceral yolk sac endoderm, parietal endoderm (Reichert's membrane), a sample of trophoblast (dissected from Reichert's membrane) and placenta. The weights of the total conceptus, the placenta and the foetus were recorded, along with the crown–rump length and morphological index based on hind limb development (McLaren & Buehr, 1990; Palmer & Burgoyne, 1991). The morphological index values were converted to a numerical scale for statistical analysis. (For example, stages 7 and late 7 were transformed to 7·0 and 7·5 respectively.) Unless the foetus was too immature, the proportion of pigmented cells in the retinal pigment epithelium of each eye was subjectively estimated and averaged.

After removal of the foetal heads (for another study), the foetus and placenta were stored at −20 °C, in 100 μl of 50% glycerol in water, in 1·5 ml microtubes. All other tissues were stored in 10 μl of 50% glycerol in microtest plates. Samples were lysed by three cycles of freeze/thawing with mechanical disruption of the foetal and placental tissues. Electrophoresis, staining for glucose phosphate isomerase (GPI-1) activity and quantification of the %GPI-1A by scanning densitometry were carried out as previously described (West & Flockhart, 1994). Maternal tissue (e.g. in placentas) produced only the GPI-1C enzyme and was excluded from the analysis of electrophoresis bands. GPI-1AB heteropolymer was produced by some chimaeric placentas (West, Flockhart & Keighren, 1995). Half of %GPI-1AB hetero-

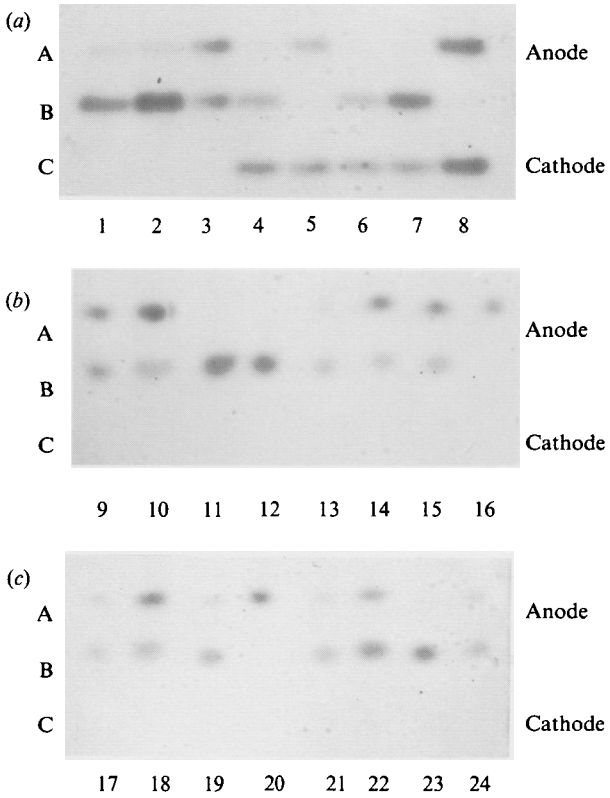

Fig. 1. GPI-1 electrophoresis plates; samples were loaded either with a 0·25 μl applicator (*a*) or a fine Pasteur pipette (*b–c*). GPI-1 allozymes (A, B and C) are indicated at the side; migration was towards the cathode. (*a*) lanes 1–3, foetus from XP-35, 36 and 37; lanes 4–8, placenta from XP-11, 12, 13, 14 and 15 (maternal GPI-1C present); (*b*) lanes 9–16, amnion from XP-19, 20, 21, 22, 23, 24, 25 and 26; (*c*) lanes 17–24, parietal endoderm from XP-1, 2, 3, 4, 5, 6, 7 and 8. Some of the minor bands that were detectable on the original electrophoresis plates and scans are not visible in the photograph.

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Table 3. *%GPI-1A (or % albino in the eye) in tissues of 12½ day chimaeric conceptuses in series XP ($AF_1 \times BALB/c \leftrightarrow BF_2$), ranked by %GPI-1A in the foetus*

Chimaera ref.	Primitive ectoderm lineage*				Primitive endoderm		Trophectoderm	
	Eye % albino	Foetus	Amnion	YS Mes.	YS End.	P. End.	Troph.	Placenta
XP22	0	0	0	2	23	18	0	0
XP32	0	4	9	6	18	16	0	0
XP5	8	8	12	24	23	22	—	1
XP36	5	8	11	15	62	31	3	0
XP33	8	10	13	9	48	97	100	91
XP35	10	10	12	17	59	85	31	23
XP3	5	12	8	15	41	22	0	0
XP11	—	14	14	11	5	0	10	8
XP7	10	15	19	11	0	5	49	73
XP23	10	16	19	11	23	51	0	23
XP29	50	24	22	20	16	35	0	0
XP10	15	25	26	33	33	38	0	6
XP9	30	35	25	31	46	64	—	5
XP14	40	37	45	36	37	40	0	0
XP2	25	37	43	48	51	57	18	26
XP13	—	39	27	32	0	85	0	0
XP6	35	43	43	28	16	31	81	81
XP37	55	50	48	51	49	84	29	29
XP1	55	55	54	49	30	36	100	79
XP18	65	61	47	42	26	23	100	83
XP25	45	67	61	64	71	81	14	20
XP30	80	71	51	51	70	78	59	18
XP16	65	72	53	50	15	60	—	12
XP20	95	77	64	55	0	10	66	76
XP15	70	78	75	74	22	20	100	96
XP19	90	83	56	75	58	82	94	60
XP4	93	85	76	77	62	100	17	35
XP24	85	91	70	73	69	100	55	84
XP12	95	92	87	87	49	36	100	100
XP8	100	100	100	92	16	29	51	83
XP26	100	100	87	88	60	100	72	91
XP27	100	100	100	100	100	100	5	44
Mean	48.08	47.40	43.00	43.10	37.40	51.20	39.70	39.00
S.E.	6.60	5.90	5.10	5.10	4.40	5.70	7.30	6.50
S.D.	36.17	33.30	28.90	28.70	24.70	32.30	39.30	36.90
N	30	32	32	32	32	32	29	32
Coeff. Var.	75.22	70.30	67.20	66.70	66.10	63.10	98.90	94.60

* The tissues are arranged according to their developmental origin from the primitive ectoderm (epiblast), primitive endoderm (hypoblast) or trophectoderm lineage. Abbreviations: YS Mes., yolk sac mesoderm; YS End., yolk sac endoderm; P. End., parietal endoderm; Troph., trophoblast overlying Reichert's membrane; Coeff. Var., coefficient of variation (%). A further five conceptuses were non-chimaeric. Four (XP17, XP21, XP28 & XP31) were uniformly 100% GPI-1B and one (XP34) was uniformly 100% GPI-1A.

polymer was added to both %GPI-1A and %GPI-1B values, so that the %GPI-1A was calculated as $(A + AB/2) \times 100/(A + AB + B)$. The raw data (as %GPI-1A given to one decimal percentage point) was used for statistical analysis and plotting the figures but they were rounded to the nearest integer for presentation in the Tables.

(iii) *Statistical analysis*

Statistical tests were performed on an Apple Macintosh computer using statistical packages 'StatView 4.1' (Abacus Concepts Inc., Berkeley, USA) and 'MultiStat' (Biosoft, Cambridge, UK) and a routine established on Microsoft Excel (Microsoft Corporation).

3. Results

(i) *Composition of different tissues in the chimaeric conceptuses*

Two series of chimaeric conceptuses (XP and XN) were produced for comparison with two previous series as indicated in Table 2. Electrophoresis of GPI-1 was used to estimate the contribution of each of the original 8-cell stage embryos to the foetus, placenta and other extraembryonic tissues of each 12½ day chimaeric conceptuses as shown in Fig. 1. The

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Table 4. *%GPI-1A (or % albino in the eye) in tissues of 12½ day chimaeric conceptuses in series XN (BALB/c × AF_1 ↔ BF_2), ranked by %GPI-1A in the foetus*

Chimaera ref.	Primitive ectoderm lineage*				Primitive endoderm		Trophectoderm	
	Eye % albino	Foetus	Amnion	YS Mes.	YS End.	P. End.	Troph.	Placenta
XN1	0	0	0	0	11	0	0	0
XN6	0	0	0	0	0	14	0	0
XN36	0	0	0	0	9	4	0	0
XN39	0	0	0	10	0	0	85	88
XN22	5	5	8	13	23	33	32	3
XN34	3	6	6	10	0	0	0	0
XN24	0	9	14	9	54	—	1	0
XN26	0	11	10	16	12	17	0	5
XN33	20	14	23	20	23	8	55	83
XN29	10	17	15	15	47	45	5	6
XN4	25	20	32	35	9	9	12	35
XN32	15	23	25	20	37	40	100	73
XN35	35	23	11	16	30	10	0	0
XN9	15	23	26	33	19	14	38	36
XN11	15	28	33	23	19	20	53	29
XN28	20	33	29	26	57	100	0	3
XN37	35	36	30	31	36	10	0	0
XN25	45	37	47	42	17	0	75	50
XN20	15	40	29	37	9	26	11	40
XN31	25	42	28	22	0	11	0	3
XN27	40	54	54	41	35	47	14	8
XN12	55	59	53	45	28	9	95	78
XN16	55	61	34	34	71	69	0	11
XN21	75	63	54	56	87	75	30	23
XN18	70	68	77	52	51	44	100	82
XN19	55	69	44	51	58	88	—	5
XN17	65	71	72	64	41	89	31	47
XN23	85	76	59	58	42	15	11	2
XN30	85	86	87	94	89	100	81	100
Mean	29·91	33·55	31·07	30·01	31·40	31·90	29·58	27·94
S.E.	5·14	4·90	4·46	4·05	4·65	6·17	6·77	6·14
S.D.	27·70	26·40	24·03	21·81	25·02	32·67	35·82	33·06
N	29	29	29	29	29	28	28	29
Coeff. Var.	92·59	78·70	77·34	72·67	79·67	102·43	121·12	118·35

* See Table 3 for abbreviations.

A further 11 conceptuses were non-chimaeric. Nine (XN2, XN3, XN5, XN7, XN10, XN13, XN14, XN38 & XN40) were uniformly 100% GPI-1B and two (XN15 & XN41) were uniformly 100% GPI-1A. One chimaera (XN8) was dead and had completely pigmented eyes (0% albino), 0% GPI-1A in the fetus, amnion, yolk sac mesoderm and parietal endoderm, 24% GPI-1A in the yolk sac endoderm, 70% in the trophoblast sample and 53% in the placenta.

contribution of the (AF_1 × BALB/c) and (BALB/c × AF_1) cells was estimated as the %GPI-1A.

The detailed quantitative results are shown in Tables 3 and 4 for series XP and XN respectively. Data for conceptuses that were non-chimaeric in all tissues assayed and one dead conceptus are included in the footnotes to these Tables but they were excluded from Figs 2 and 3 and the statistical analysis (below). For some of the conceptus shown in Tables 3 and 4, the data are incomplete. The % pigmentation in the eye was not estimated for immature foetuses and the mixed sample of trophoblast and decidua overlying Reichert's membrane was sometimes exclusively maternal decidua (GPI-1C). Technical losses account for the other missing data.

The %GPI-1A was very significantly positively correlated among tissues within each of the three primary developmental lineages (analysis not shown) as reported for several other series of chimaeras (West, Bücher, Linke & Dünnwald, 1984; James, Flockhart, Keighren & West, 1993; West & Flockhart, 1994). Any other correlations (among tissues from different developmental lineages) were generally weaker. These included some significant positive correlations between the primitive ectoderm and trophectoderm lineages in series XP and between the primitive ectoderm and primitive endoderm lineages in series XN.

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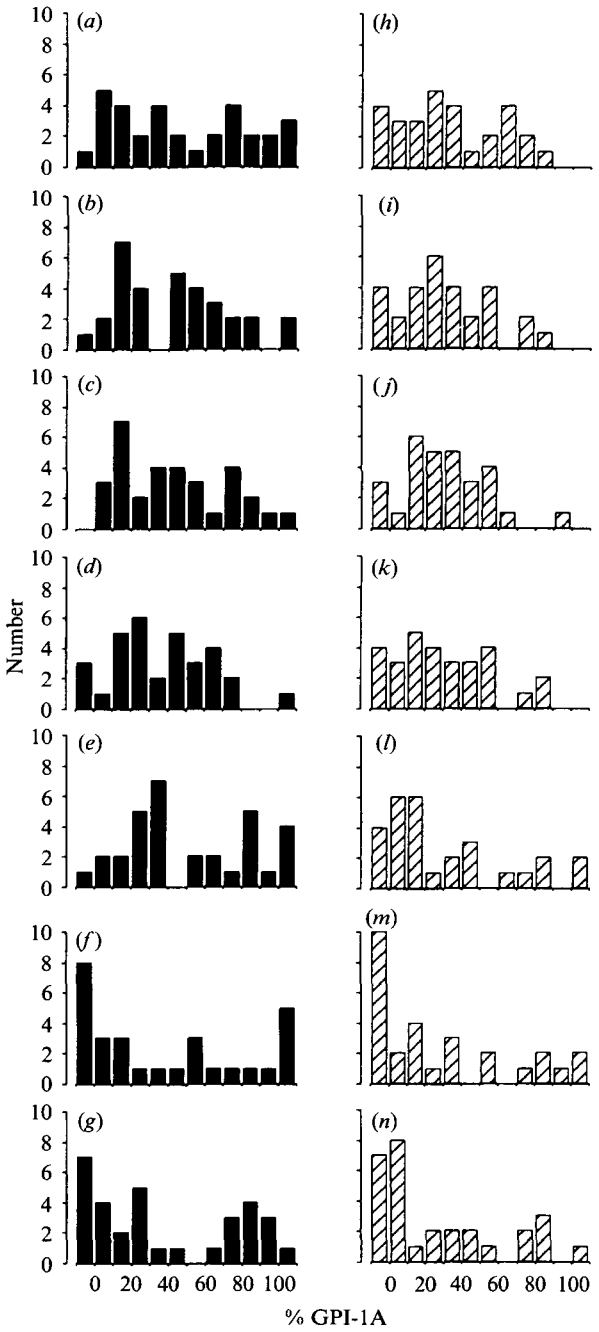

Fig. 2. Distributions of %GPI-1A in seven tissues analysed in each series of chimaeric conceptuses (XP and XN). Tissues with either 0 or 100% GPI-1A are shown separately at either end of the distributions. (*a*), XP foetus, (*b*), XP amnion; (*c*) XP yolk sac mesoderm; (*d*), XP yolk sac endoderm; (*e*), XP parietal endoderm; (*f*), XP trophoblast; (*g*), XP placenta; (*h*), XN foetus; (*i*), XN amnion; (*j*), XN yolk sac mesoderm; (*k*), XN yolk sac endoderm; (*l*), XN parietal endoderm; (*m*), XN trophoblast; (*n*), XN placenta.

(ii) *Differences in contributions of* ($AF_1 \times BALB/c$) *and* ($BALB/c \times AF_1$) *embryos to chimaeras*

The mean %GPI-1A (% albino in the eye) in the chimaeric conceptuses (Tables 3 and 4) shows that ($AF_1 \times$ BALB/c) and BF_2 cells were fairly equally represented in all eight tissues studied from the ($AF_1 \times$ BALB/c) $\leftrightarrow BF_2$ chimaeras (series XP in Table 3). However, in the (BALB/c $\times AF_1$) $\leftrightarrow BF_2$ chimaeras (series XN in Table 4), the reciprocal backcross (BALB/c mother $\times AF_1$ father) component tended to be under-represented in each tissue and typically contributed about 30%. This difference in mean %GPI-1A between the two series of chimaeras, involving reciprocal backcross embryos, is an important observation and suggests involvement of a maternal genetic effect, as discussed below.

In series XP, five conceptuses (13·5%) failed to show chimaerism in any of the tissues analysed; one was entirely GPI-1A and four were entirely GPI-1B. In series XN, 11 conceptuses (27·5%) failed to show chimaerism in any of the tissues analysed; two were entirely GPI-1A and nine were entirely GPI-1B. Excluding the dead chimaera, the frequency of non-chimaeric conceptuses was not significantly higher in series XN (11/40 versus 5/37; $\chi^2 = 1{\cdot}51$; $P = 0{\cdot}22$). However, the 2:9 ratio (100% GPI-1A: 100% GPI-1B) in series XN was significantly different from the 1:1 ratio expected by chance ($\chi^2 = 4{\cdot}45$; $P < 0{\cdot}05$), whereas the 1:4 ratio in series XP was not significant ($\chi^2 = 1{\cdot}80$).

The data in Tables 3 and 4 are ranked by %GPI-1A in the foetus and it can be seen that for series XN (Table 4) most (20/29; 69%) of the foetuses have less than 50% GPI-1A whereas for series XP (Table 3), this proportion is lower. This, together with the differences in the means and the significant preponderance of 100% GPI-1B non-chimaeric XN conceptuses, suggests that the composition of the (BALB/c $\times AF_1$) $\leftrightarrow BF_2$ chimaeras (XN) differs significantly from that of series XP and may be unbalanced in favour of the BF_2 component. The histograms in Fig. 2 also suggest that, while the distributions are reasonably balanced for series XP, they tend to be moderately skewed in favour of the BF_2 component (low GPI-1A) in series XN.

To decide whether series XN was genotypically unbalanced, we considered each of the seven tissues analysed by GPI-1 electrophoresis separately and grouped the chimaeras into two sets of classes, as previously described (West & Flockhart, 1994). The first classification (Ia in Table 5) was based on that used by Mullen & Whitten (1971). Individual samples were divided into three classes according to the %GPI-1A: < 30, 30–70 and > 70%. If the number of individuals in the 30–70% GPI-1A class was not the highest or joint highest of the three classes, the strain combination was considered to be atypical. As already noted by Falconer & Avery (1978), the distributions of the compositions of chimaeric tissues are often flat rather than bell-shaped. This was true, for example, of the %GPI-1A in the foetuses of series XP (Fig. 2*a*). To increase the discrimination between the bimodal distributions, typical of trophoblast and placenta (James *et al.* 1993; West & Flockhart, 1994), and the flat or bell-shaped distributions typical of other tissues, the thresholds of the first classification system were

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Table 5. *Chimaeric conceptuses grouped according to %GPI-1A in each tissue (three classifications shown)*

Tissue	Classification of distribution of GPI-1A: Ia < 30:30–70: > 70	Ib < 25:25–75: > 75	II < 50: > 50	Statistical significance† χ^2 value
Balanced distributions (according to classification II)				
XP Foetus‡	12:9:11	11:12:9	18:14	0·50
XP Amnion‡	14:12:6	11:15:6	19:13	1·12
XP Yolk sac mesoderm	12:12:8	11:16:5	20:12	2·00
XP Parietal endoderm	10:11:11	9:12:11	17:15	0·13
XP Trophoblast‡§	15:6:8	14:8:7	17:12	0·86
XP Placenta‡§	18:3:11	16:6:10	20:12	2·00
Unbalanced distributions (according to classification II)				
XP Yolk sac endoderm	15:14:3	13:18:1	22:10	4·50*
XN Foetus	15:11:3	14:13:2	20:9	4·17*
XN Amnion	16:10:3	12:15:2	22:7	7·76**
XN Yolk sac mesoderm	15:13:1	14:14:1	23:6	9·97**
XN Yolk sac endoderm	16:10:3	14:13:2	22:7	7·76**
XN Parietal endoderm	17:6:5	16:8:4	22:6	9·14**
XN Trophoblast	17:5:6	16:6:6	20:8	5·14*
XN Placenta	18:5:6	17:7:5	22:6	9·14**

* $P \leqslant 0{\cdot}05$; ** $P \leqslant 0{\cdot}01$.
† Tested against the expectation of equal proportions of < 50% and > 50% GPI-1A in classification II.
‡ Classified as atypical by classification Ia because there were fewer individuals with 30–70% GPI-1A than in one of the other categories.
§ Classified as atypical by classification Ib because there were fewer individuals with 25–75% GPI-1A than in one of the other categories.

altered to < 25, 25–75 and > 75% (classification Ib in Table 5).

In the second classification (II in Table 5), the strain combination was considered to be unbalanced if the number of individuals with < 50% GPI-1A was statistically significantly different from the number with > 50% GPI-1A. Using these two criteria, the distributions of %GPI-1A in different tissues were classified as (1) balanced and typical, (2) balanced but atypical or (3) unbalanced (classification systems Ib and II in Table 5). Applying the above criteria, only the yolk sac endoderm from series XP was considered unbalanced, the trophoblast and placenta samples were classified as balanced by 'atypical' and the other tissues in this series were all balanced and typical. In contrast, all of the tissues from series XN were classified as unbalanced, indicating that the $(BALB/c \times AF_1) \leftrightarrow BF_2$ strain combination was genotypically unbalanced. Although the differences in %GPI-1A between series XP and XN were statistically significant for most tissues when the < 50%: > 50% ratio was tested against the expected 1:1 ratio, only the parietal endoderm shows a significant difference when the < 50%: > 50% ratios in XP and XN were tested against each other ($\chi^2 = 4{\cdot}25$; $P = 0{\cdot}039$).

(iii) *Evidence for a maternal genetic effect on the chimaeric composition*

The above comparisons indicate that, when aggregated to BF_2 embryos, embryos from the reciprocal backcrosses $(BALB/c \times AF_1)$ and $(AF_1 \times BALB/c)$ made different mean contributions to all chimaeric tissues examined but this difference was relatively modest. The mean % albino in the eyes and %GPI-1A in the other seven tissues analysed are shown in Fig. 3 for $(BALB/c \times AF_1) \leftrightarrow BF_2$ and $(AF_1 \times BALB/c) \leftrightarrow BF_2$ chimaeras as well as the $BALB/c \leftrightarrow BF_2$ and $AF_2 \leftrightarrow BF_2$ chimaeras analysed previously (West & Flockhart, 1994). The mean contribution of both AF_2 and $(AF_1 \times BALB/c)$ cells, in series XM and XP respectively, was about 45% in most tissues whereas the contribution of BALB/c cells in series XR was much lower (mostly 15–20%) and that of $(BALB/c \times AF_1)$ intermediate at about 30%. Thus, although both $(BALB/c \times AF_1) \leftrightarrow BF_2$ and $BALB/c \leftrightarrow BF_2$ chimaeras were classified as unbalanced (Table 5 and West & Flockhart, 1994), the imbalance was more pronounced in the $BALB/c \leftrightarrow BF_2$ chimaeras.

Table 6 indicates that, for each tissue analysed, the %GPI-1A (or % albino in the eyes) varies significantly among the four series of chimaeras studied. Results of pairwise statistical comparisons are given in the legend to Fig. 3. These show that the %GPI-1A clearly differs significantly between series XR and XM but the difference between the reciprocal backcross chimaeras (XN and XP) only reached statistical significance for the parietal endoderm (as with the χ^2 tests on the < 50%: > 50% ratios, above). However, series XN and XP do differ significantly in their relationships with series XR and XM. For most tissues, the intermediate contribution of $(BALB/c \times$

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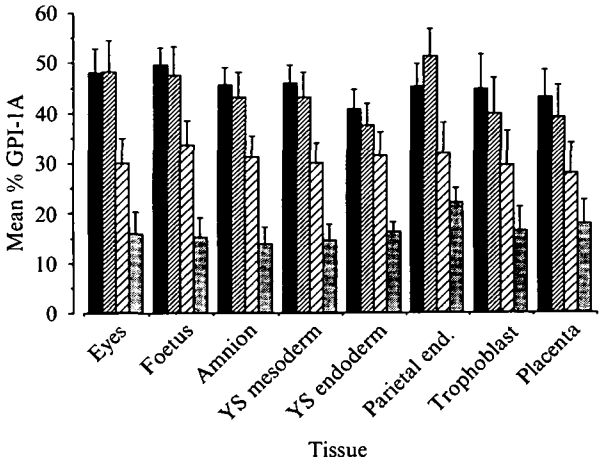

Fig. 3. Histogram comparing the mean %GPI-1A (or % albino in the eyes) in eight tissues of chimaeric conceptuses from series XM, XP, XN and XR. Pairwise comparisons by Mann–Whitney *U*-tests showed that the GPI-1A contribution to chimaeras in series XR (BALB/c ↔ BF_2) was very significantly lower ($P \leqslant 0{\cdot}001$) than that in both series XM and series XP in all eight tissues. It was also significantly lower ($P \leqslant 0{\cdot}05$) than that in series XN for all tissues, except the parietal endoderm ($P = 0{\cdot}398$). The %GPI-1A in series XM ($AF_2 \leftrightarrow BF_2$) did not differ significantly from that in series XP ($AF_1 \times$ BALB/c ↔ BF_2) in any tissue but it was significantly higher ($P \leqslant 0{\cdot}04$) in six tissues (all but the yolk sac endoderm and trophoblast) from series XN (BALB/c × $AF_1 \leftrightarrow BF_2$). The contribution of GPI-1A in the two series of chimaeras involving reciprocal backcross embryos (series XN and XP) only differed significantly for the parietal endoderm ($P = 0{\cdot}012$). ■, XM: ($AF_1 \times AF_1$) ↔ BF_2; ▨, XP: ($AF_1 \times$ BALB/c) ↔ BF_2; ▨, XN: (BALB/c × AF_1) ↔ BF_2; ▨, XR: (BALB/c × BALB/c) ↔ BF_2.

AF_1) cells (series XN) was significantly different from both that of (BALB/c × BALB/c) and ($AF_1 \times AF_1$) cells (series XR and XM respectively). In contrast, the contribution of cells from the reciprocal backcross ($AF_1 \times$ BALB/c) embryos in series XP consistently differed very significantly from that of (BALB/c × BALB/c) but not from that of ($AF_1 \times AF_1$) cells. These relationships imply that embryos from BALB/c mothers contributed significantly less to the chimaeras than those from AF_1 mothers. Comparison of various physical parameters (Table 6) revealed few significant differences, although series XM had the heaviest and most advanced conceptuses.

(iv) *Viability of chimaeras from different series*

If the unbalanced tissue compositions seen in series XR and XN were the result of lower viability of chimaeras with significant contributions of BALB/c or (BALB/c × AF_1) cells, there should be significantly more embryonic lethality among these two series of chimaeras than among the two balanced series. Table 7 shows that there was some variation, among the four series of chimaeras, in the proportion of aggregated embryos that implanted (37–51 %) and the proportion of implantations that survived to form normal $12\frac{1}{2}$ day foetuses (58–90 %). However, the overall frequency of preimplantation embryo losses was lower in the two unbalanced series (49 %) than in the two balanced series (58 %) and the proportion of postimplantation embryo losses was not significantly higher [40/138 (29 %) versus 26/104 (25 %); $\chi^2 = 0{\cdot}538$; $P = 0{\cdot}463$]. There is, therefore, no evidence that chimaeras with significant contributions of BALB/c or (BALB/c × AF_1) cells are less viable.

(v) *Sources of experimental variation in chimaeric composition*

Although the above differences in composition of the four groups of chimaeras are likely to reflect genetic differences in mouse strain combinations, there are several possible sources of experimental variation that may also influence the composition of chimaeras. It can be seen from Tables 3 and 4 that the composition of individual chimaeras within a series varies widely. Thus, the mean %GPI-1A in two small series of chimaeras could differ by chance alone. Also, different batches of chimaeras of the same strain combination could differ in unknown systematic ways. For example, batches of preimplantation embryos may be exposed to different conditions during chimaera production and those collected from different females may vary slightly in developmental stage.

The chimaeras in series XP (Table 3) provided an opportunity to investigate variation among batches. All 32 chimaeras in this series were produced in three batches, with each batch being produced on a different day. There were 10 chimaeric conceptuses from two litters in batch 1 (XP1-XP10), 12 chimaeras (plus two non-chimaeras) from five litters in batch 2 (XP11-XP24) and 10 chimaeras (plus three non-chimaeras) from five litters in batch 3 (XP25-XP37). Comparison of the composition of the eight chimaeric tissues produced in these three batches showed little variation between batches and only that for the yolk sac endoderm reached statistical significance in the Kruskal–Wallis test ($P = 0{\cdot}025$). Thus, the level of variation among the three batches of series XP was considerably lower than that found among different series of chimaeras by the same statistical test ($P \leqslant 0{\cdot}001$ for all eight tissues tested; Table 6). The variation among batches was not consistent for all eight tissues; the highest mean %GPI-1A in the yolk sac endoderm and parietal endoderm occurred in batch 3 whereas for all other tissues the highest mean value occurred in batch 2.

Another possible source of experimental variation is GPI-1 electrophoresis and quantification. The foetus and placenta samples of the four series of chimaeras analysed in the first experiment (either the present study or that reported by West & Flockhart, 1994) were also analysed separately in a second experiment (West *et al.* 1995). In this second experiment, the foetus

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Table 6. *Comparison of %GPI-1A (or % albino in the eye) in different tissues and physical parameters of four groups of 12½ day chimaeric conceptuses*

	Series XR (BALB/c × BALB/c) Mean ± S.E. N = 38†	Series XN (BALB/c × AF_1) Mean ± S.E. N = 29†	Series XP (AF_1 × BALB/c) Mean ± S.E. N = 32†	Series XM (AF_1 × AF_1) Mean ± S.E. N = 33†	Statistical variation among different series	
					P_{KW}‡	P_A‡
Composition of chimaeric tissue						
Eyes (% albino)	15·8 ± 4·4	29·9 ± 5·1	48·1 ± 6·6	47·9 ± 4·9	< 0·0001*	NA
Foetus	15·1 ± 3·9	33·6 ± 4·9	47·4 ± 5·9	49·5 ± 3·7	< 0·0001*	NA
Amnion	13·7 ± 3·7	31·1 ± 4·5	43·0 ± 5·1	45·6 ± 3·6	< 0·0001*	NA
YS Mesoderm	14·3 ± 3·5	30·0 ± 4·0	43·1 ± 5·1	45·9 ± 3·7	< 0·0001*	NA
YS Endoderm	16·0 ± 2·2	31·4 ± 4·6	37·4 ± 4·4	40·6 ± 4·1	< 0·0001*	NA
Parietal End.	22·0 ± 3·0	31·9 ± 6·2	51·2 ± 5·7	45·1 ± 4·7	0·0002*	NA
Trophoblast	16·3 ± 4·9	29·6 ± 6·8	39·7 ± 7·3	44·7 ± 7·0	0·001*	NA
Placenta	17·6 ± 5·1	27·9 ± 6·1	39·0 ± 6·5	43·0 ± 5·7	< 0·0001*	NA
Weights and other physical parameters						
Conceptus (mg)	312·1 ± 7·2	327·3 ± 10·5	307·3 ± 8·9	330·2 ± 9·5	0·147	0·201
Foetus (mg)	101·8 ± 3·4	98·1 ± 4·4	93·1 ± 4·2	107·9 ± 3·5	0·053	0·057
Placenta (mg)	85·5 ± 2·3	89·7 ± 2·3	83·0 ± 2·6	93·0 ± 2·6	0·027*	0·027*
Foetus/placenta ratio	1·20 ± 0·04	1·09 ± 0·04	1·12 ± 0·05	1·16 ± 0·03	0·333	0·243
Crown–rump (mm)	9·30 ± 0·10	9·18 ± 0·18	9·07 ± 0·22	9·17 ± 0·11	0·687	0·766
Hind limb score	7·03 ± 0·11	7·16 ± 0·17	7·13 ± 0·14	7·45 ± 0·12	0·011*	NA

* The variation among groups was considered to be significant when $P \leqslant 0{\cdot}05$.
† Missing data: in series XR, N (eyes) = 34 and N (trophoblast) = 37. In series XN, N (parietal endoderm) and N (trophoblast) = 28. In series XP, N (eyes) = 30, N (trophoblast) = 29 and N (hind limb) = 31. In series XM, (yolk sac mesoderm) and N (yolk sac endoderm) = 32 and N (trophoblast) = 28.
‡ P_{KW} values from non-parametric Kruskal–Wallis test; P_A values from 1-way factorial analysis of variance with 3 degrees of freedom. Student's *t*-tests showed that the placentas in series XM were significantly heavier than in series XP ($P = 0{\cdot}009$) and series XR ($P = 0{\cdot}034$). Mann–Whitney *U*-tests showed that the hind limb morphology was significantly more advanced in series XM than in series XR ($P = 0{\cdot}001$). Results of pairwise statistical tests comparing the composition of the chimaeric tissues are given in the footnote to Fig. 3.
NA, Not applicable.

Table 7. *Viability of balanced and unbalanced series of chimaeras at 12½ days*

	Number (%)			
	Balanced series		Unbalanced series	
	XM	XP	XN	XR
Aggregated embryos transferred†	138	111	140	131
Females with implantations	14	12	31	19
Total number of implantations	63 (46%)	41 (37%)	71 (51%)	67 (51%)
Resorbing moles	22	4	30	10
Normal foetuses	41 (65%)*	37 (90%)*	41 (58%)*	57 (85%)*

* Expressed as a percentage of total implantations.
† Excluding those transferred to females with no implantation sites.

and placenta from the same chimaera were run in adjacent tracks on the same electrophoresis plate. (In the first experiment, reported here, batches of the same type of tissue from different chimaeras were run together.) This provided an opportunity to investigate variation in the estimation of the %GPI-1A. For three of the eight sets of samples, the mean %GPI-1A was higher in the first experiment and for the other five sets it was higher in the second experiment. The paired mean values from these two experiments were very similar (all within 4% of each other) and paired comparisons with the Wilcoxon signed rank test revealed only one significant difference between experiments. The mean %GPI-1A in XM foetuses was 49·5 ± 3·7 in expt 1 and 51·3 ± 3·7 in Expt 2. Although these two sets of data differed significantly ($P = 0{\cdot}009$) the difference between the means was less than 2%. For other pairs of samples, the *P*-values were much higher and none was significant (range: $P = 0{\cdot}221$–$0{\cdot}711$). Assay variation, therefore, seems unlikely to be a significant source of experimental variation.

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These two sets of comparisons revealed no experimental variation that would significantly influence the estimated %GPI-1A in the chimaeras. This strengthens the conclusion that observed variation is largely attributable to differences between the embryos from different matings.

(vi) *Comparisons of preimplantation development*

Preimplantation embryos from different crosses were compared, to test for differences in developmental rate that could underlie the differences in compositions of the four groups of chimaeras. Subjective observations made during the production of chimaeras with (BALB/c × BALB/c) embryos suggested that these lagged behind BF_2 embryos at $2\frac{1}{2}$ days (J. H. Flockhart, unpublished). Also, more systematic observations showed that (BALB/c × AF_1) embryos were retarded compared to (AF_1 × AF_1) embryos collected at $2\frac{1}{2}$ and $3\frac{1}{2}$ days. At $2\frac{1}{2}$ days, all 162 (AF_1 × AF_1) embryos were beginning to compact, compared with 66/79 (BALB/c × AF_1) embryos ($\chi^2 = 25{\cdot}05$; $P < 0{\cdot}0001$). At $3\frac{1}{2}$ days, 71/75 (AF_1 × AF_1) embryos had reached the blastocyst stage compared with 77/105 (BALB/c × AF_1) embryos ($\chi^2 = 12{\cdot}20$; $P = 0{\cdot}0005$). These preliminary results are compatible with the possibility that slow rates of preimplantation development of (BALB/c × AF_1) and (BALB/c × BALB/c) embryos underlie the poor contributions of these embryos to chimaeric conceptuses. However, comparisons of *in vitro* preimplantation development produced less consistent results (data not shown).

4. Discussion

The results imply that embryos from BALB/c mothers contribute less to the tissues of mid-gestation chimaeric conceptuses than embryos from AF_1 mothers. Although other genetic factors are clearly operating, because inbred (BALB/c × BALB/c) embryos make a significantly poorer contribution than backcross (BALB/c × AF_1) embryos, a maternal genetic effect (matrocliny) is implicated.

Support for the conclusion that embryos from BALB/c mothers contribute less well to chimaeras than embryos from AF_1 mothers has also been obtained from another two series of chimaeras (J. D. West, M. Keighren and J. H. Flockhart, unpublished). These chimaeras were of the strain combinations (BALB/c × CMA) ↔ BF_2 and (AF_1 × CMA) ↔ BF_2, where CMA is a non-inbred, transgenic stock derived from crosses between the partly congenic strain C57BL/Ola.AKR-*Gpi-1s*^{a},*c*/Ws and strain 83 of Lo (1983, 1986). Preliminary analysis of eight tissues from $12\frac{1}{2}$ day conceptuses showed that the mean (BALB/c × CMA) contribution was consistently lower than the (AF_1 × CMA) contribution in the other series (33–43% versus 44–58% GPI-1A).

The unbalanced phenotypes of BALB/c ↔ BF_2 and (BALB/c × AF_1) ↔ BF_2 chimaeras seen in every tissue analysed could be a result of (1) death of chimaeras with significant contributions of BALB/c or (BALB/c × AF_1) cells, (2) generalized cell selection against cells derived from embryos with BALB/c mothers or (3) preferential allocation of BALB/c and (BALB/c × AF_1) cells to the mural trophectoderm, which makes little contribution to the $12\frac{1}{2}$ day conceptus. Our comparison of embryonic losses in four different series of chimaeras rules out the first of these possibilities.

Four developmental lineages are established prior to implantation in the late blastocyst stage (Gardner & Papaioannou, 1975; fig. 6 in West & Flockhart, 1994). After the inner cell mass (ICM) and trophectoderm become distinguishable, the ICM divides into the primitive ectoderm and primitive endoderm lineages. The surrounding trophectoderm can be divided into polar trophectoderm (adjacent to the ICM) and mural trophectoderm (adjacent to the blastocoelic cavity). While the primitive ectoderm, primitive endoderm and polar trophectoderm all contribute substantially to the $12\frac{1}{2}$ day conceptus (see Table 3), the mural trophectoderm cells cease division and produce only a small number of primary trophoblast giant cells (Copp, 1978, 1979). None of the $12\frac{1}{2}$ day tissues analysed would have contained a significant contribution of mural trophectoderm derivatives, so our analysis cannot test whether BALB/c and (BALB/c × AF_1) cells are allocated preferentially to the mural trophectoderm lineage (possibility 3 above). Further studies on younger chimaeras are, therefore, needed to distinguish between possibilities (2) and (3).

Maternal effects in mammals may be transmitted through the germline (cytoplasmic or chromosomal) or via somatic cells (through the mother's milk or the environment of the reproductive tract), as discussed by McLaren (1981). The observed maternal effect on mid-gestation chimaeric composition cannot be transmitted via milk or the uterine environment (because chimaeric blastocysts were all transferred to pseudopregnant females of the same CF_1 hybrid strain). However, it may be mediated by an effect of the reproductive tract that acted before the embryos were flushed from the oviduct at the 8-cell stage. Alternatively the effect could be a consequence of cytoplasmic inheritance or genomic imprinting. Without further studies it is not possible to distinguish between these various possibilities.

Since 4-cell stage embryos contribute poorly to the inner cell mass (ICM) when aggregated to 8-cell stage embryos of the same age (Spindle, 1982), a maternal effect that retarded preimplantation development of BALB/c and (BALB/c × AF_1) embryos might cause these embryos to make a lower contribution to the ICM than to the trophectoderm lineage at the blastocyst stage. Two further possibilities are discussed

below. First, the preimplantation embryos that develop slowly may also contribute poorly to the derivatives of polar trophectoderm. Second, that BALB/c and (BALB/c × AF_1) embryos develop relatively slowly and that a maternal effect may be partly responsible. Together, these suggest a mechanism whereby BALB/c or (BALB/c × AF_1) cells are likely to be preferentially allocated to the mural trophectoderm, as in possibility (3) above. However, as already noted, studies of earlier stage chimaeric embryos will be necessary to test this possibility.

First, unless a lagging BALB/c or (BALB/c × AF_1) embryo was preferentially allocated to the mural trophectoderm, when aggregated with a BF_2 embryo, it would still colonize the polar trophectoderm and so contribute to the $12\frac{1}{2}$ day trophoblast and placenta. The BALB/c or (BALB/c × AF_1) trophectoderm cells would either have to segregate preferentially to the mural region of the trophectoderm layer, by some unknown mechanism, or become displaced to this region by movement of ICM cells (predominantly BF_2 cells) into the overlying polar trophectoderm. The polar trophectoderm lineage could then become predominantly populated by BF_2 cells, like the primitive ectoderm and primitive endoderm. This remains purely speculative but there is evidence to support the idea that some polar trophectoderm cells are derived from the underlying ICM cells in the blastocyst (Handyside, 1978; Edirisinghe, Wales & Pike, 1984; Cruz & Pedersen, 1985; Handyside & Hunter, 1986; Dyce, George, Goodhall & Fleming, 1987; Winkel & Pedersen, 1988).

Second, there is evidence that BALB/c embryos develop more slowly than those from some other strains and the paternal and maternal BALB/c genomes appear to each play a role in retarding preimplantation development. Whitten & Dagg (1961) showed that the second cleavage division occurred later in (BALB/cGn × BALB/cGn) inbred preimplantation embryos than in (BALB/cGn × 129/Rr)F_1 hybrid embryos. Similarly, Shire & Whitten (1980*a*) demonstrated that the first cleavage of [(SJL × SWR)F_1 female × BALB/cWt male] embryos was delayed compared to (SJL × SWR)F_2 embryos. This could be attributable to early acting embryonic genes or to an effect of the paternal genome on, for example, the timing of mating or fertilization. An effect of the paternal or embryonic BALB/c genome is consistent with our observation that (BALB/c × BALB/c) embryos made a lower contribution to chimaeras than (BALB/c × AF_1) embryos.

Our chimaera experiments implied that there was a more significant maternal effect because (BALB/c female × AF_1 male) embryos made a lower contribution to chimaeras than (AF_1 female × BALB/c male) embryos. An effect of the maternal BALB/c genotype on preimplantation development was reported by Shire & Whitten (1980*b*). The median time to first cleavage was about 2·7 h later in (BALB/cBy × BALB/cWt) embryos than in (C57BL/6By × BALB/cWt) or [(C57 × BALB/c)F_1 female × BALB/cWt male] embryos. The cleavage time of eggs from (C57 × BALB/c)F_1 females was not more variable, so the authors argued that this was not likely to be an effect of early acting embryonic genes. (Greater variability would be expected if cleavage time was affected by segregation of C57 and BALB/c alleles from the F_1 females.) Our own preliminary observations on (BALB/c × BALB/c), (BALB/c × AF_1) and (AF_1 × AF_1) preimplantation embryos support the idea that a BALB/c maternal effect retards development.

McLaren & Bowman (1973) found that a maternal effect was responsible for slow development of inbred C3H/BiMcl and (C3H/BiMcl female × C57BL/Mcl male)F_1 preimplantation embryos, which lagged behind C57BL/Mcl inbred and (C57BL/Mcl female × C3H/BiMcl male)F_1 embryos. This maternal effect was subsequently attributed to delayed fertilization in C3H/BiMcl strain females (Nicol & McLaren, 1974). It is not known whether fertilization is similarly delayed in BALB/c females but this would explain why Shire & Whitten (1980*b*) found a maternal effect as early as the first cleavage.

In conclusion, our experiments have revealed that a significant part of the difference in composition between the four groups of chimaeras is attributable to a maternal genetic effect of some kind. This maternal effect must act before the 8-cell stage but it is not yet known whether it is mediated via cytoplasmic inheritance, genomic imprinting or the reproductive tract. Similarly, it is not known whether this maternal effect causes a generalized selection against BALB/c and (BALB/c × AF_1) cells in the chimaera or whether it causes these cells to be preferentially allocated to the mural trophectoderm lineage in the blastocyst. The evidence that a maternal effect retards preimplantation development of embryos from BALB/c females leads us to speculate that this might cause them to contribute poorly to chimaeric conceptuses when aggregated with more precociously developing embryos. Further experiments are needed to test these possibilities.

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