GENETIC STUDIES OF THE SYRIAN HAMSTER I. THE MUTANT GENES CREAM, RUBY-EYE AND PIEBALD

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Introduction

Since the capture of living specimens of the Syrian hamster (*Mesocricetus auratus*) in 1930 (Adler and Theodor 1931, Bruce and Hindle 1934, Adler 1948) the hamster has become a well-known laboratory animal. The hamster has also been acclaimed as a domestic pet, thousands of animals being produced annually by fanciers. The mounting increase in number of hamsters produced, either in the laboratory or by fanciers, implies that the probability of detection of mutations may be increased. This paper records the occurrence of two mutations which have appeared in Britain and presents an investigation of possible linkage between the two mutations and a third imported from the United States. The possibility of partial sex-linkage has also been examined. The two mutations have been noted briefly when their mode of inheritance had been determined (Robinson 1955).

Description of mutations

A short account of the pigmentation of the normal golden hamster will be given, followed by a description of the mutant types.

Normal

The pelage of the normal hamster is composed of relatively soft hairs and is appreciably denser on the dorsum than on the ventral-lateral surfaces of the body. Dorsal coat colour is largely golden-brown, richer along the mid-dorsal line than on the flanks. When the fur is parted, the hairs can be seen to be slate blue sub-apically, becoming paler towards the skin. The fur of the under-surface of the body tends to be pale creamy-white with a pale-grey base. A variable white patch is occasionally present in the region of the umbilicus. The sparse hair on the feet and on the stubby tail is very light cream. The ears are large and the skin is darkly pigmented while the hair covering is scanty and cream-white in colour. The under-surface of the jaw and the two throat "flashes" are cream-coloured and are connected with the belly colour by a thin cream line separating the light golden chest-band. Anterior to the second flash is a dark curving streak of melanin pattern known as the "crescent". Melanic pigment forms in the skin of the ears and along the edges of the eyelid. Pigment also forms diffusely in the dermis of the scrotum and as spots on the prepuce of males; and diffusely

in the perineal region of females. The two hip-glands are surrounded by short, apparently coarser, hairs, darker in colour than the ordinary fur. Examination of the reverse side of pelts indicate that pigment is present in the skin about the two glands. The area and amount of gland pigmentation is more pronounced in adult males than in females.

Pigment can be seen macroscopically in the skin of the ears from the age of about five days (transient pigment in the skin of the body commences from the second day) whereas the initial signs of pigment in the genital region are delayed until about the eighteenth day. In females the perineal pigment is diffuse, but in males two characteristic laterally placed spots on the prepuce can be discerned. In the skin, pigmentation increases with age but at different rates and with some variation of extent in different animals. Particularly, the number of spots on the prepuce may increase in both number and irregularity. The pelage colouring appears to be relatively stable upon attainment of maturity. The juvenile hamster is "mouse" grey in colour, the golden-brown pigmentation developing with subsequent waves of newly erupting hair.

Non-extension of melanin

Animals homozygous for non-extension of melanin possess rich creamy yellow fur, somewhat paler on the stomach than on the dorsum. This mutant type is known colloquially as the cream. No melanin appears to develop in the pelage, except possibly within the stiff hairs associated with the hip gland. Melanin, however, develops normally in the skin of the ears and rim of the eye-lids, in the region of the perineum according to the sex of the individual, and in the hip gland. The difference in degree of gland pigmentation between the sexes, noted in the normal, is more readily apparent. The coarse hairs of the hip-gland appear to be slightly more richly coloured (light orange) than the ordinary fur. Similarly to the normal hamster, the ears, lower parts of the feet, and tail are thinly covered with creamy-white hairs. No melanin formation in the skin of the body can be seen from the day of birth onwards. This fact enables the cream to be accurately distinguished from the normal at the age of two days should this be necessary. It has been noticed that although the rate of pigmentation of the ears parallels that of the normal, the pigment may be laid down irregularly. The erect ears viewed against a strong light appear to be "marbled". Later, the lighter pigmented areas darken and eventually the ears are usually as evenly coloured as in the normal. The simplest explanation for the coat colour would be that of inhibition of melanin from the fur. It would appear that the intensity of yellow is less in the cream than in the normal. It is an interesting speculation whether this is an artifice of the inhibition of melanin or if the yellow pigmentation *per se* has been diluted. The rate of growth, the viability of both sexes, and the fertility of each appear comparable to the normal. The mutation was discovered by a fancier in 1951 at Leeds. The mutant allele is completely recessive to the normal and the symbol **e** is proposed for its designation.

Ruby-eye dilute

The coat colour of the ruby-eye animal is distinctly weakened; the black is diluted to bluish and the yellow to fawn. The sub-apical undercolour of the pelage is a medium blue becoming greyish towards the roots of the hair. Fur colour on the ventral surfaces of the body is very pale. Eye colour is also affected, the pupil assuming a rich ruby mien in most, but exceptionally dull lights. Of the black or yellow pigmentation, the black appears to be more strongly diluted. New born animals display an annulus of iris pigmentation, clearly visible beneath the closed eyelids. The reduced pigmentation of the eye distinguishes the ruby-eye individual from either the normal or the cream. The rate of development of transitory pigmentation in the skin of young nestlings is retarded. The difference is apparent macroscopically by the fifth day from birth. Melanin formation in the skin is severely weakened, ear colour is peach grey, and little or no pigment is present about the genitals of either sex. Hip-gland pigmentation is correspondingly reduced, although several months later accumulation of pigment may be evident. At 21 days of age the ruby-eye hamster is significantly smaller in size than the normal and the size reduction persists into the adult stage. Ruby-eye dilution is due to a completely recessive mutation for which the symbol **ru** is proposed. The mutation was observed by a fancier at Southsea in the year 1948.

The reduction in size is accompanied by impaired viability, and probably general stamina, with a sterility onset in the male. An attempt to estimate the viability of **ru ru** animals surviving at 21 days is made complex by inviability interactions between the **ru** allele and the two other mutations. Of the three crosses, only the second and third classes of cross I (Table 5) would appear to yield an estimate the viability of **ru ru** animals at 21 days, unbiased by known interaction or linkage. The viability based upon these 136 animals is 0.5814 ± 0.1034 and is highly significant. The sexes differ slightly for viability (males 0.6765 ± 0.1827 and females 0.5192 ± 0.1232) but the difference (0.1573 ± 0.2203) is within its sampling error.

Evidence for sterility in the male ruby-eye is not as extensive as could be desired but suggestive notwithstanding. During 1954 two young adult males (12-weeks and 16-weeks, respectively) were obtained from different sources and repeatedly mated with five females. All matings were negative and four of the females subsequently produced litters by other males. Two males bred in the colony (1954), were each penned from birth with a little sister until 12 weeks of age. No young were produced during this period although the final four weeks (at least) could have been productive. Both females subsequently produced litters by other males. In an independent experiment, conducted during 1955, three females were housed continuously from birth with two males for seven, eight and ten weeks respectively, but no young were produced. All three females subsequently gave birth to litters by normal males. A scrutiny was maintained for signs of pregnancy in addition to actual parturition. Over the same interval, 1954 to 1955, 40 non-ruby-eye males, maintained under identical conditions of diet and husbandry, were found to be fertile. Sexual compulsion (interest in female genitals, eagerness and persistency of copulation) appears to be undiminished in both

E-ru ru and **e e ru ru** animals. Finally, four hamster fanciers have stated that between them forty-four males have been tested for fertility (circa 1950-1955) but without success. The adequacy of the test for each of the 44 males is unknown (it may be interjected that probably few of these animals were utilized for breeding at a sufficiently early age since it is usual practice among fanciers to defer breeding from immature animals; see below). Sterility among normal males in the general population is so low as to scarcely stimulate comment. However, during 1956 several fanciers reported that litters had been obtained from immature ruby-eye males and the question was re-investigated.

A more detailed study of the condition is at present being carried out at the National Institute for Medical Research by Miss H. M. Bruce. Her preliminary findings, from the 10 males so far examined, are in agreement with the general observations mentioned above. All 10 males were fertile at puberty but only for a short time. Sex drive did not appear to be affected, but fertile matings were rare after about 3 months of age (see Table 1). As judged by the appearance of the vaginal smear taken from the females within a few hours of copulation, spermatogenesis in the infertile males was markedly reduced. Confirmation of this was found from histological examination of the testes of one male killed at 154 days of age. A full report of this work will be given elsewhere.

Table 1. *Fertility of matings by ruby-eye males*

No. of males examined	Age at copulation (days)	Total No. of matings	No. of litters resulting	♀ examined for egg penetration		Fertility rate (%)
				Total No.	No. having penetrated eggs	
10	<90	25	16			64
10	90–150	43	2*	5	1	5–7§
4	>150	33	0			0

* sired at 97 and 141 days of age respectively by different males, the latter after a succession of infertile matings.

§7% if presence of penetrated eggs be regarded as resulting from a fertile mating.

Bruce's findings necessitated further study of the failure to produce young under the environment of previous years. In the autumn of 1956, five **ru ru** males were each housed separately with a female. All five pairs produced a litter within the span of 7-11 weeks. The difference in performance over the years could be due to chance (the probability of occurrence of this particular configuration, of four sterile prior to 1955 and five fertile later, in a 2×2 table is $P = 0.0079$, by Fisher's exact method) or to an increase in the duration of fertility; or even to the recent appearance of fertility in the male ruby-eye. Both **E-ru ru** and **e e ru ru** animals were found to be equally fertile.

The recombination of the two mutations **e** and **ru** has resulted in a new colour type, the ruby-eye cream. The **e e ru ru** phenotype is paler in colour than **e e Ru-**. Fur colour is pastel cream, with a distinct absence of the "yellow" tone found in the dark-eyed cream. A reduction appears to occur in the intensity of pigmentation of the ear pinna. Ruby-eye animals of genotype **E-ru ru** have peach-grey ears (with some variation)

while the ear colour of **e e ru ru** is very pale, with many individuals appearing devoid of pigment.

Both ruby-eye males and females are more nervous and excitable than the normal. They are easily disturbed and frightened by unaccustomed noises. An apparently extended interval is required for either to become tractable to routine handling by an attendant. Probably as a consequence of this, the rate of loss of whole litters is slightly higher among ruby-eye mothers, although otherwise fecundity is quite good. Foote and Foote (1950) have noted that the piebald female is nervous and more prone to neglect its young. This is confirmed among the piebalds here, whose nervousness exceeds that of the ruby-eye. In contradiction to the ruby-eye and piebald, the cream is placid. With all three mutants, and in general, the males are more tractable than the females. All the animals received similar treatment of handling.

Piebald

A recessive type of white spotting has been described in America by Foote (1949) and Foote and Foote (1950), and also by Orsini (1952). It is probable that the separate occurrences are due to the segregation of the same gene. These studies disclose that the two white-spotted or piebald hamsters share common features of growth retardation and certain urinogenital anomalies. The distribution of white spotting varies considerably between animals but displays one or two general idiosyncrasies. The first appearance of the spotting is in the region of the chest and throat. As the piebald pattern becomes more extensive the white spotting spreads slowly over the whole ventral areas of the stomach and jaw. A streak of white appears from the nose, extending between the eyes. The whole of the face may be white, with the exception of coloured areas surrounding the eye and ears. These are the last two sectors of the head to become involved in the pattern. In animals with higher grades of piebald, the spotting reaches the shoulders and begins to appear as spots on the lumbar regions of the body. This may be seen rather vividly in figure 8A of Orsini's paper. These foci increase in area and coalesce until a belt of pigmented fur remains, transversing the back. The last vestiges of pigmentation are usually confined to the ears and hindmost regions of the body. These tendencies are general, it must be emphasized, for the pattern is highly irregular and may be extensively broken up, with considerable brindling of white and coloured hairs. Highgrade piebalds may have the eye bulbus (one or both) situated within a depigmented volume of tissue and when this occurs, the pupil often assumes a reddish glint. The piebald stock of this paper, upon which the above description is based, are descended from piebalds imported from America during 1950.

The data tabulated by Foote and Foote (1950) for body weight attained at 7-day intervals suggest that the smaller size of piebalds is due to a reduced rate of growth. Piebald animals appear to require more considerate handling than the normal or other mutant types. Short falls or mishandling, which normally would not harm, could result in bone fractures with the piebald. Whether this is due to their lighter skeleton structure (as a consequence of the smaller overall body size) or to a defective skeleton is unknown but would repay study. The latter possibility is worthy of consideration

in view of the known mal-plasia of some soft tissues. Skeleton fragility is even more marked in the **ru ru s s** animal.

The present form of white spotting is due to the segregation of a single recessive gene, for which the symbol **s** is proposed. Examination of the frequency of segregation of the piebald gene in crosses II and III indicate a significant deficiency of piebald homozygotes. An unbiased estimate of the degree of viability of piebald homozygotes at 21 days would appear impossible due to possible linkage between the genes **ru** and **s**, or an inviability interaction between the two which results in a higher than expected death of **ru ru s s** zygotes. An unbiased estimate is available from Orsini's data and is 0.4898 $\pm$0.1220. If genetic independence is assumed between the genes **ru** and **s**, then estimates from the two appropriate phenotypic classes of the viability of piebald for the two crosses are 0.4663$\pm$0.0915 and 0.4273$\pm$0.0666, respectively. These values do not differ significantly from those obtained from Orsini's data. In both crosses the male sex has the lower viability (0.4021$\pm$0.1187 and 0.3129$\pm$0.1381, respectively) compared with that for the female (0.5313$\pm$0.1398 and 0.5389$\pm$0.1697).

Koch (1951) has reported briefly upon an inherited white spotting in the hamster which has recently appeared in Germany. The white spotting is stated to be inherited as a recessive mutation and would appear to resemble phenotypically the spotting associated with the present gene. It is not clear if the gene is a new spontaneous mutation or if animals heterozygous for piebald may have been imported into Germany.

Weight differences

To examine the extent of differences in weight between the various phenotypes described in this paper, together with the influence of sex, a sample number of individuals of each phenotype were chosen. Consecutive litters were selected subject to the condition that the rarer phenotypes were well represented within each litter. This was an attempt to reduce the amount of inter-litter variation of weaning weight on the one hand, and to secure reasonably large numbers of the rarer occurring phenotypes, on the other. Both very large and abnormally small litters were ignored. It is possible that this method of sampling of individuals may accentuate the degree of weight difference between phenotypes, due to the "enhancement effect" described by Beatty (1956) and to intra-litter competition for milk supply. However, since young hamsters tend to consume solid food at an early age (from 10 days onwards), the degree of accentuation may be slight. In spite of the method, the number of animals of phenotype **E-ru ru s s** are inadequate and no representatives of class **e e ru ru s s** were secured. All weights were obtained at the weaning age of 21 days, and the mean weights for each phenotype are brought together by table 2.

The presence of varying numbers of individuals within the fourteen classes indicates that the data are not orthogonal. This has necessitated an analysis of variance, as described by Quenouille (1950; §7A 9) and summarised by Table 3. The primary source of variation is that due to phenotype. The sum of squares ascribable to the sex difference barely exceeds the 5% level of significance. Ignoring the sex difference, it is obvious that the phenotypes fall into four groups, associated with the genes **ru**, **s**, and

the gene combination **ru ru s s.** The successive decreases in 21-day weight are in approximate harmony with the decreases in relative genetic viability. An interaction between phenotype and sex is not supported by the analysis. The coefficients of variation (C.V., Table 2) for each class are relatively stable over the various phenotypes, with the exception of the last two. This may be due to the low sample number or to the enhanced debility of the combination **ru ru s s.** Elimination of the sum of squares due to the weight difference between sexes yields an adjusted analysis of variance differing only slightly from that of table 3. The variation between phenotypes is highly significant.

Item	*d.f*	*S.S.*	*m.s.*
Between phenotypes	6	3920.68	653.45
Within phenotypes	295	4720.38	16.00
	301	8645.06	

The adjusted (for varying proportions within phenotypic classes) mean sex difference of weight, 0.939 ± 0.460, is just significant. The sex difference is consistently manifested over all phenotypes. Those phenotypic differences of interest are tabulated in table 4. The frequency distribution for the main groups of phenotypes are diagrammed by figure 1. It may be noted, as a point of subsidiary relevance, that the weight distribu-

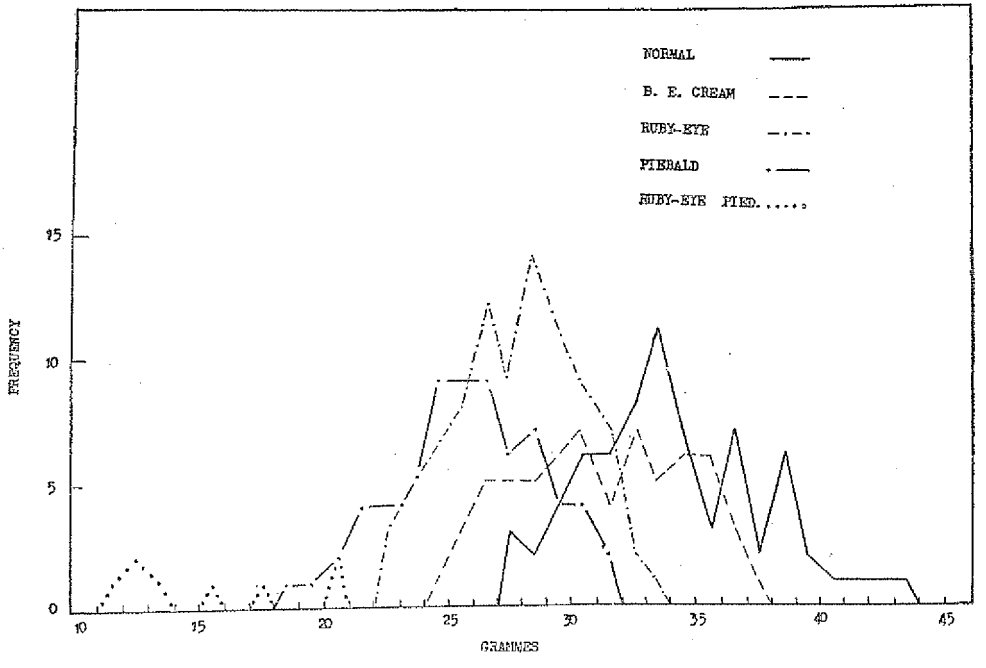

Figure 1. Distribution of 21-day weight for different phenotypes

tion of **e e Ru-S-** animals covers a range somewhat lower than those of the normal hamster. The smallest are approximately 3g. lighter than the smallest of the normals

while the largest are some 6g. lighter than the largest of the normals. The difference (2.734±0.691) is highly significant, but the generality of a real difference in 21-days weight between **E-** and **e e** individuals may be queried, since an identical trend is not significant between **E- ru ru S-** and **e e ru ru S-**; indeed, it is reversed for **E- Ru- s s** and **e e Ru- s s** phenotypes (see table 2).

The mean weight tabulated here for the normal hamster appears to be comparable with the 21-day weight shown by the graph on the growth chart given by Poiley (1950). On the other hand, the mean weights of both normal and piebald are presumably significantly larger than those given by Foote and Foote (1950) for their colony of normal (27.39g.) and piebald (21.63g.) hamsters. However, the weight reduction attendant with piebald is of similar proportions in the two stocks. Expressing the mean of the piebald as a percentage of the normal, yields two similar values, namely, 76% (Robinson) and 79% (Foote and Foote). Orsini (1952) reports an average weight of 20.5g. for her piebald animals, which agrees with the observations of Foote and Foote.

Table 2. *Mean weights of young at 21 days of age for different phenotypes*

Phenotype	Sex	Mean (g.)	C.V.	% of normal	No.
E–Ru–S–	♂	32.84±0.70	2.14	100	32
	♀	33.18±0.89	2.67	100	39
e e Ru–S–	♂	29.12±1.10	3.78	89	34
	♀	31.47±0.62	1.96	95	30
E–ru ru S	♂	27.11±0.46	1.71	83	28
	♀	27.36±0.61	2.24	83	28
e e ru ru S–	♂	27.10±0.58	2.14	83	20
	♀	27.35±0.67	2.45	82	17
E–Ru–s s	♂	24.24.±0.68	2.80	73	21
	♀	25.67±0.80	3.10	77	18
e e Ru–s s	♂	25.74±0.96	3.74	78	15
	♀	26.62±0.58	2.19	80	13
E–ru ru s s	♂	14.00±1.00	7.14	43	2
	♀	15.33±2.91	18.99	46	6

Table 3. *Analysis of variance of 21-day weight*

Item	*d.f*	*S.S.*	*m.s.*
Phenotype	6	3915.78	652.63**
Sex	1	66.84	66.84*
Phenotype × sex	6	52.55	8.76
Residual	289	4667.83	16.15
	302	8703.00	

*significant, **highly significant

Table 4. *Mean differences between categories of phenotypes for 21-day weight (adjusted for sex)*

	Comparison		Difference (g)
E–Ru–S–	*v*	**e e– – – –**	0.630±0.467
E–Ru–S–	*v*	**– –ru ru– –**	4.423±0.540**
E–Ru–S–	*v*	**– – – – –s s**	6.233±0.597**
– –ru ru S–	*v*	**– – – –s s**	1.810±0.641**
– –ru ru S–	*v*	**E–ru ru s s**	12.497±1.479**
– – – –s s	*v*	**E–ru ru s s**	10.688±1.502**
E–Ru–S–	*v*	**e e Ru–S–**	2.734±0.691**

** highly significant.

ANALYSIS OF SIMULTANEOUS SEGREGATION

Experimental crosses

Three experimental crosses have been completed involving all the mutant genes. The essential details are collocated in tables 5 and 6, and are designated as crosses I, II and III. The main body of table 5, under phenotypic classes, tabulates the observed frequencies realized from each cross, the material being scored for coat colour and sex. The segregations of crosses II and III are partitioned, as rows, into normal (**S**) and piebald (**s**), combined with the genotypes heading the columns. The gene symbols employed are those proposed above while *Y* and *X* are adopted as representing the sex differentiating segments of the *X Y* pair of chromosomes. It is conceivable that segregation of a mutant gene may display a sex association which could be interpreted as partial sex-linkage. All the young were sexed and classified at 21 days of age. For the initial few days post-partum, some cannibalism occurs by the mother but there is no evidence that this is selective (in particular, against **ru ru** or **s s** animals).

Table 5. *Data from experimental crosses*

Cross	Mating type		E Ru		e Ru		E ru		e ru	
			♂	♀	♂	♀	♂	♀	♂	♀
I	**Y E Ru/X e ru** × **X E Ru/X e ru**		136	143	34	52	23	27	3	9
II	**Y e Ru s/X e ru S** ×	**S**	50	50	47	46	23	36	32	31
	X e ru s/X E ru S	**s**	3	9	10	8	0	1	0	1
III	**Y e Ru s/X E ru S** ×	**S**	124	129	39	38	43	29	9	15
	X e Ru s/X E ru S	**s**	12	18	5	12	1	1	0	0

Table 6. *Classification for segregation analysis*

Cross	Mating	Loci		Phenotype Classes				Total
		a	*b*	*AB*	*aB*	*Ab*	*ab*	
I	CII	**e**	**ru**	279	86	50	9	424
	CIB	**e**	**Y**	159	37	170	58	424
	CIB	**ru**	**Y**	170	26	195	33	424
II	CBI	**e**	**s**	159	156	13	19	347
	RBI	**ru**	**s**	193	122	30	2	347
	CBB	**ru**	**Y**	110	55	113	69	347
	RIB	**s**	**Y**	152	13	163	19	347
III	RII	**e**	**ru**	283	94	74	24	475
	CII	**e**	**s**	325	101	32	17	475
	RIB	**e**	**Y**	180	53	177	65	475
	RII	**ru**	**s**	330	96	47	2	475
	CIB	**ru**	**Y**	180	53	197	45	475
	RIB	**s**	**Y**	215	18	211	31	475

C=coupling, R=repulsion, I=intercross,
B=backcross, for the respective alleles.

(a) *Cross I.*

The initial crosses set up were of two types (a) **e ru** ♂♂ × **ERu** ♀♀ and (b) **ERU** ♂♂ × **e ru** ♀♀. The first was a failure, due to the sterility among ruby-eye males, while the second constitutes cross I. Full details of the segregation are given in table 6. Examination of the segregation would indicate considerable disturbance of the expected ratios. The sex distribution over the various classes appears to be at random and does not depart significantly from expected equality ($X_1^2=2.42$, $P>0.1$). Combining the observed frequencies for the sexes appropriately over the phenotypic classes, the completed analysis for segregation of the two genes is as follows:

	x^2	*d.f.*	*P*
e segregation	1.52	1	> 0.20
ru ,,	27.79	1	< 0.001
e–**ru** linkage	0.61	1	> 0.30
Total	29.92	3	< 0.001

The disturbance in the observed segregation is apparently due to inviability of a proportion of **ru ru** zygotes. An overall estimate of the viability of animals homozygous for ruby-eye is 0.4849 ± 0.0689. However, this ignores a possible inviability interaction between the genes **e** and **ru,** since examination of the segregation reveals that the observed frequency in the **e e ru ru** class falls short of expectation, even after allowance for the inviability of **ru.** An estimate of the viability of the **e e ru ru** zygote should make allowance for possible linkage between the two mutants. Accordingly, the following expectations of the phenotypic classes were constructed, where p = cross-over

fraction between **e** and **ru**, v = viability of **ru** independent of **e**, w = viability of the combination **e e ru ru**, $P = p^2$ and $D = 3+v(1-P)+vwP$.

	E Ru	**e Ru**	**E ru**	**e ru**
Expectation	$\frac{2+P}{D}$	$\frac{1-P}{D}$	$\frac{v(1-P)}{D}$	$\frac{vwP}{D}$
Observations	279	86	50	9

Simultaneous estimation of the three parameters from the maximum likelihood functions and the corresponding inverse of the information matrix yields the following values: $p = 1 - \sqrt{P} = 0.4586 \pm 0.0599$, $v = 0.5814 \pm 0.1026$ and $w = 0.4341 \pm 0.2077$. The last two estimates (of v and w) differ significantly from their expectations of unity. It is possible that the deficiency of **e e ru ru** animals is real and not a statistical deviation. However, it must be remarked the interaction has failed to manifest in crosses II and III.

Cross I yields information upon the joint segregation of the mutants **e** and **ru** in conjunction with sex. The inviability associated with the presence of the **ru** gene introduces a small complication since the inviability may not be evenly apportioned over all phenotypic classes. This is especially the case for the **e**—**Y** segregation. Putting $z = (1+vw) / (1+v)$, the following expectations may be formulated.

	EY	**EX**	**eY**	**eX**
Expectation	$\frac{2-p}{3+z}$	$\frac{1+p}{3+z}$	$\frac{zp}{3+z}$	$\frac{z(1-p)}{3+z}$
Observation	159	170	37	58

Estimation by maximum likelihood gives the values $p = 0.4341 \pm 0.0436$, with $z = 0.8663$. Analysis of the **ru** — **Y** segregation proceeds in a similar manner but with $z = v(1+w)$. The solution yields the value $p = 0.5067 \pm 0.0511$, with $z = 0.4849$.

(b) *Cross II*

The matings which engendered cross II were from an F_1 which was produced by mating cream piebald males (**YX e e Ru ru ss**) with ruby-eyed females (**XX E e ru ru S S**). From these litters matings were arranged as shown by column two of table 5, with the resultant segregation tabulated in the main section of the table. It is evident from inspection of the segregation that considerable disturbance exists. There is a deficiency of both **ru ru** and **s s** animals, as brought out by a chi-square test of the segregation of the individual mutations.

	x^2	*d.f.*	*P*
e segregation	0.026	1	< 0.80
ru „	28.245	1	> 0.001
s „	46.072	1	< 0.001
Sex „	0.833	1	> 0.30

Further inspection of table 6, in which the complete segregation is regrouped for simultaneous segregation of chosen pairs of genes, suggests an association between **ru** and **s**. Fully examined, the joint segregation of **ru** and **s** necessitates the simultaneous estimation of p, v and u, the latter representing the viability of **s s** zygotes. The following expectations may be developed for the various phenotypic classes, with $D = 1 + p + u(1 - p) + v(2 - p) + uvp$.

	Ru S	**ru S**	**Ru s**	**ru s**
Expectation	$\frac{1+p}{D}$	$\frac{v(2-p)}{D}$	$\frac{u(1-p)}{D}$	$\frac{uvp}{D}$
Observation	193	122	30	2

Estimation proceeding by maximum likelihood and by inversion of the information matrix produces the following values: $p = 0.3759 \pm 0.0710$, $v = 0.2909 \pm 0.0726$ and $u = 0.2433 \pm 0.590$. The viabilities v and u differ significantly from unity. It should be noted that the values of v and u are smaller than those yielded by apparently unbiased estimates. While the present value of p does not differ significantly from expectation, these results need to be interpreted in relation with those for the joint segregation of **ru** and **s** belonging to cross III.

With the assumption that the inviabilities associated with the presence of **ru** and **s** do not interact with each other or with **e** and sex, estimation and possible significance of the recombination fraction occurring between the three comparisons **e—s, ru—Y** and **s—Y,** may be assessed. The expectations corresponding to the phenotypic classes for simultaneous segregation of **e** and **s** may be found as under; giving the estimates $u = 0.3048$, $p = 0.4478 \pm 0.0611$.

	E S	**e S**	**E s**	**e s**
Expectation	$\frac{2-p}{3+u}$	$\frac{1+p}{3+u}$	$\frac{up}{3+u}$	$\frac{u(1-p)}{3+u}$
Observation	159	156	13	19

The independency test for segregation of **ru** and *Y* reduces to a double backcross, with a similar inviability associated with two complementary classes. The recombination fraction is computed as $p = 0.4624 \pm 0.0413$, with $v = 0.5561$. Examination of the segregation of *Y* and **s,** leads to similar expectations as **s** and **e** but of opposite linkage phase. Estimation of p for the pair of alleles gives 0.5149 ± 0.0611.

(c) *Cross III*

The third series of crosses were produced by the same initial matings as for cross II. The matings were instituted by the author and the F_2 were raised and classified by Mr. G. T. C. Gore. Examination of the segregation immediately discloses that certain of the expected ratios are disturbed.

	χ^2	*d.f.*	*P*
e segregation	0.006	1	> 0.90
ru „	4.834	1	< 0.05
s „	54.625	1	< 0.001
Sex „	0.171	1	> 0.50

The most interesting comparison is that of the independency of **ru** and **s**. Arranging of the observations from line eight of table 6 as a 2×2 table, and applying Yates' correction for continuity, a χ^2_c of 8·047 is realized; a highly significant result and one which is supported by a parallel tendency in the corresponding segregation of cross II. Biometrically, the frequencies of occurrence of animals in the four classes are not independent. Two explanations may by proposed for these observations. Genetic linkage may occur between the genes, effectively reducing the frequency of classes **Ru S** and **ru s**; or an inviability interaction may exist between the two mutants, effectively reducing the frequency of class **ru s**. Assuming the validity of the linkage explanation and independency of the impaired viability of each mutant, the following expectations may be formulated, with $D = 2+p^2+(u+v)(1-p^2)+uvp^2$.

	Ru S	**ru S**	**Ru s**	**ru s**
Expectation	$\frac{2+p^2}{D}$	$\frac{v(1-p^2)}{D}$	$\frac{u(1-p^2)}{D}$	$\frac{uvp^2}{D}$
Observation	330	96	47	2

Simultaneous estimation leads to the values $p = 0{\cdot}2497 \pm 0{\cdot}0784$, $v = 0{\cdot}6399 \pm 0{\cdot}0781$ and $u = 0{\cdot}3133 \pm 0{\cdot}0485$. All three parameters differ significantly from their expected values and the one of interest, p, has a probability of $P < 0{\cdot}01$.

The alternative explanation is that of independent segregation but with an inviability interaction between the mutants additional to the usual inviability. If the interaction viability is denoted as x, four parameters are now postulated. With three degrees of freedom, one of these must be taken as fixed. In the present case, this is $p = 0{\cdot}5$. The expectations are quickly formed ($D = 9+3u+3v+xuv$).

	Ru S	**ru S**	**Ru s**	**ru s**
Expectations	$\frac{9}{D}$	$\frac{3v}{D}$	$\frac{3u}{D}$	$\frac{xvu}{D}$

Formulae for estimating v, u and x were derived, the sampling variance in each case being

$$\sum \left\{ a \left(\frac{\delta T}{\delta a} \right)^2 \right\} - N \left(\frac{\delta T}{\delta N} \right).$$

The estimated values are $v = 0{\cdot}8727 \pm 0{\cdot}1012$, $u = 0{\cdot}2849 \pm 0{\cdot}0630$ and $x = 0{\cdot}1463 \pm 0{\cdot}1070$. The relative values of these viability estimates appear to deserve comment. That for v is considerably larger than values found for the other segregation and that for u conversely lower; while the interaction viability is very low (merely 4% of the phenotype surviving). One reason for these values could be that the phenotypes **Ru**± **s s** and **ru ru s s** (particularly the latter) were selectively perishing in face of intra-uterine competition. One obvious effect of this could be to increase the proportion of **ru ru S-** which survive to term. Selection falling more heavily upon the **s s** foetuses could ease selection pressure against **ru ru** foetuses.

A supporting item in favour of selective degeneration is the absence of an inviability interaction between the genes **e** and **ru** noted in cross I but absent in crosses II and III. With the present data, no decision can be made between the two alternative explanations of the disturbed **ru** and **s** joint segregation. However, it is proposed to set up an identical mating but with the genes **ru** and **s** in opposite linkage phase. In the absence of further complications, the results of this proposed cross should clarify the underlying mechanism.

We may consider briefly the remaining five cases of simultaneous segregation appearing in this cross. These are relatively uncomplicated. The **e**—**ru** comparison has the following expectations ($P = p^2$).

	E Ru	**e Ru**	**E ru**	**e ru**
Expectation	$\frac{2+P}{3+v}$	$\frac{1-P}{3+v}$	$\frac{v(1-P)}{3+v}$	$\frac{vP}{3+v}$
Observation	283	94	74	24

This yields an estimate of $p = 0{\cdot}4880 \pm 0{\cdot}0322$ with $v = 0{\cdot}7798$. The comparison **e**—**s** has a similar expectation to the above but of opposite linkage phase ($P=(1-p)^2$) and an inviability u associated with the segregation of the **s** allele. The estimate of p is $0{\cdot}4317 \pm 0{\cdot}0441$, with $u = 0{\cdot}3451$. The three mutants may be compared with the segregation of sex. The data for the pair **e**—Y furnishes an estimate for p of $0{\cdot}5111 \pm 0{\cdot}0397$. The distribution of the four classes for **ru**—Y and **s**—Y are disturbed by the differential viabilities but the expectations can be formulated. These are similar to those of **e**—Y in cross I; allowance being made for the relevant linkage phase. For **ru**—Y, $p=0{\cdot}5489 \pm 0{\cdot}0423$, with $v=0{\cdot}7798$ and for **s**—Y, $p=0{\cdot}5074 \pm 0{\cdot}0509$, with $u=0.3571$.

Combination of data

The precision of the various estimates of the recombination fraction between mutant alleles may be increased by suitable amalgamation of groups of segregation data. This has been achieved by maximum-likelihood scores, and the conclusions are summarised in table 7. The χ_1^2 values suggest that none of the segregation for the listed pairs of genes provide evidence for the existence of genetic linkage, after allowance is made for differential viability. The table omits one of the six possible comparisons with three mutants and sex (maleness being treated genetically as a dominant gene). The exception is the comparison **ru**—**s**, where the segregation is such as to admit of linkage but the evidence is equivocal.

Several supplementary items are appended in the table. Column six provides an index for the concept of phase balance, conveniently shown as the percentage of the total amount of information contributed by a segregation in coupling linkage phase. Perfect balance is shown by the index 50, while 100 or 0 infers that all the data are in coupling or repulsion, respectively. Groups of data in perfect phase balance possess the implication that certain inviability interactions which could pass unnoticed are, on the average, cancelled out. Column eight shows the closest linkage compatible

with the collated data and derived as $0{\cdot}5 - 1{\cdot}96s$. In the table, the standard error (s) of p, the total score (S) and amount of information (I) are computed for $p = 0{\cdot}5$.

Table 7. *Summary of extent of linkage tests*

Loci	p	s	S	I	Phase Balance	χ^2_1	Closest linkage
e–ru	0.488	0.032	−11.5556	962.3515	22	0.139	0.437
e–s	0.437	0.036	−49.1110	781.7775	100	3.085	0.430
e–Y	0.493	0.029	− 8.6667	1159.5414	45	0.065	0.442
ru–Y	0.505	0.024	8.0000	1542.6387	100	0.042	0.450
s–Y	0.551	0.039	33.3333	653.3332	0	1.701	0.423

PRENATAL LOSS

It is evident from the segregation data that some prenatal loss is associated with the genes **ru** and **s**, individually or together. Now a deficiency of zygotes with these genes probably derives from the interplay of several factors. Gametes bearing the mutant allele may be more likely to perish relative to those not bearing the genes. Fusion achieved, the zygote homozygous for either gene (and both) may succumb at any stage prior to parturition while the possible hazards of parturition could take a heavier toll. Finally, the possibility of selective cannibalism by the mother is not impossible, should the **ru ru** or **s s** nestling be unduly sluggish in movement, or be of smaller size.

Study of the distribution of pre-natal loss is undoubtedly more satisfactory than indirect statistical analysis. Direct scrutiny has the disadvantage, however, that it is not always possible to determine whether the missing or degenerative foetuses are largely concentrated within certain phenotypes. Essentially the same results are obtained by examination of the young, with less trouble and without sacrificing the female. Intra-uterine study may sort out several of the factors concerned without providing positive evidence of the part played by the genetic constitution of the foetus, *per se*, or of the mother in conjunction with the foetus. Hammond's (1928) conclusions stress that foetal atrophy is primary determined by the mother. That the foetal genotype could determine which particular foetus may degenerate at a given moment for an expected total rate of foetal loss is admissible but not easy of demonstration. However, it would appear that the present crosses in the hamster, where potentially "susceptible" foetuses occur in different proportions, may be capable of providing statistical evidence in lieu of direct study. The segregation data of the present crosses are significantly disturbed, and the simplest explanation for this would be that certain zygotes are dying because of their genotype *per se*. However, a more inclusive hypothesis would be that the rate of foetal loss acts selectively upon certain genotypes. The relation would probably be one of inter-dependence, the rate of foetal loss being partially modified by the proportion of 'susceptible' genotypes. Statistical analysis pre-supposes that the proportion of foetal atrophy is concentrated within the susceptible classes. Clearly, this may not be so, in which case the proportion of foetal loss is underestimated.

The statistic (F) representing proportion of foetal loss is readily found from the expectations of two phenotypic categories, (i) those not displaying significant reductions from expected ratios between themselves and (ii) those which do so. The standard is, therefore, deviation from Mendelian expectation and the statistic, if in error for absolute proportion, is less in error, conceivably, for the ratio of foetal loss between the two categories. The statistic and its variance are,

$$F=\frac{a-lb}{(l+1)a} \qquad V(F)=\frac{l^2b(a+b)}{a^3(l+1)^2}$$

where l is expected ratio of the first category to the second, and a and b are the observed numbers in each.

Application of the formula to the three crosses yields the following results. In cross I the last four phenotypes of table 5 are significantly reduced, consequently $l = 3$, and $F = 0{\cdot}129 \pm 0{\cdot}017$. For cross II, those classes involving **ru** and **s** are below expectation, $l = 0{\cdot}6$ and $F = 0{\cdot}326 \pm 0{\cdot}032$. Finally in cross III, identical classes are reduced and $l = 1{\cdot}286$ and $F = 0{\cdot}190 + 0{\cdot}024$. All three estimates are formally significant and an inverse trend is apparent between the values of l and F. This would indicate either the interdependence or, more probably, the greater importance of foetal genotype for prenatal loss. The trend in F should imply heterogeneity between the estimates and this is examined by the analysis of table 8. The heterogeneity between crosses for the overall estimate F of 0·157 is highly significant. A negative correlation between F and l is thus *prima facie* established. Further evidence in agreement with this trend may be found in the data of Orsini (1952). Orsini records the disturbed segregation of 49 **S s** and 24 **s s** from backcross matings of **S s** with **s s**, thus $l = 1$ and $F = 0{\cdot}255 \pm 0{\cdot}061$. Incorporating Orsini's data into the test of heterogeneity yields a combined estimate of F of 0·159 and heterogeneity $\chi^2_3 = 24{\cdot}52$ ($P < 0{\cdot}001$).

Table 9 exhibits the association between proportion of prenatal loss and the ratio of normal to deficient phenotypic classes. The ordinary contingency χ^2_8 for the three crosses this paper is equal to 87·7 ($P < 0{\cdot}001$). It is of interest to consider if the obvious trend in the figures could be adequately described as linear. The method of Armitage (1955) may be utilized to isolate the linear trend component and has the value $\chi^2_1 = 84{\cdot}24$ ($P < 0{\cdot}001$). For this test, the scaling scores adopted were the actual values of l. Table 9 shows a similar analysis but incorporating the data of Orsini. The probable relationship between F and l may be regarded as linear upon the strength of this data.

Table 8. *Analysis of prenatal loss between crosses* ($F = 0.157$)

Cross	χ^2	*d.f.*	P
I	3.45	1	> 0.05
II	17.50	1	< 0.001
III	1.70	1	> 0.10
Heterogeneity	22.65	2	< 0.001

Table 9. *Association between prenatal loss (F) and segregation ratio (l); see text*

l	0.6	1	1.286	3
F	0.326	0.255	0.190	0.129

	χ^2	*d.f.*	*P*
Linear trend	83.915	1	< 0.001
Deviation	3.950	2	> 0.1
Total	87.865	3	< 0.001

DISCUSSION

The acceptance of the Syrian hamster as a useful addition to the existing range of laboratory rodents scarcely needs comment. Its utility as an experimental animal has been recognised (Poiley 1950). However, linkage analysis has to wait upon the discovery of mutations. It is to be presumed that mutations will come forward in a possibly steady, if haphazard, trickle, as in other laboratory rodents. One aspect of hamster genetics to be emphasized is the ready publication of complete segregations which can be utilized for linkage analysis. As yet, it would appear that little data is available but this state of affairs will alter. In some species the full publication of genetic segregation data has not always been considered of sufficient value for permanent record. If the segregation is such, however, that the data can be utilized for linkage studies, these views no longer hold. Statistical methods are now available by which such data may contribute information to a common analysis. The adoption of maximum likelihood scores enables data to be amalgamated, even if composed of diverse mating types or linkage phases. By this means greater reliability can be attached to statements of supposedly independent segregation or to a calculated recombination fraction.

Utilization of the convenience afforded by maximum-likelihood scoring has produced two surveys of the genetic literature (Carter and Falconer (1952) for the mouse and Robinson (1956) for the rabbit), where scattered groups of segregation data have been systematically combined. Reviews such as these permit the assessment of quasi-independent segregation between the numerous gene comparisons and reveal deficiencies (if such exist) among the comparisons. Some of these deficiencies could probably be rectified if the value of certain segregations were realized at the time and published. The main deficiency brought out by these reviews was the unawareness of the possibility of partial sex-linkage.

Robinson (1956) has discussed several of the difficulties associated with linkage investigation among pairs of genes. The labour involved in collecting linkage data is subject to diminishing returns and beyond a certain stage must be considered unrewarding. However, this disadvantage can be partially circumscribed by making the collection of data on presumably independent genes additional to other formal genetic work. In particular, the systematic testing of a new mutation usually involves the simultaneous segregation of 'known independent genes'. For the expenditure of a small effort in fore-planning useful information could be gathered and merged with existing material.

Since the formal genetics of the Syrian hamster is in its infancy, it is urged that the

presentation of research which involves gene segregation amenable to linkage investigation be published in full for future collations. The information may be compactly arranged in the style of table 5 if pressure of other work prevents immediate analysis.

SUMMARY

Three mutations in the Syrian hamster are described (cream, ruby-eye and piebald) and the six possible comparisons between the three mutants and sex have been studied for genetic linkage. No evidence for linkage could be found for five comparisons. The sixth comparison (ruby-eye-piebald) exhibited an aberrant segregation which could result from autosomal linkage or from an inviability interaction between the two mutants concerned. Two mutants (ruby-eye, piebald) display significant prenatal inviability and an attempt has been made to elucidate the nature of the prenatal loss. The hypothesis is discussed that the inviabilities observed are a consequence of an inter-dependence between "normal" foetal atrophy and the expected Mendelian proportion of susceptible phenotypes. Since hamster genetics is in its infancy—a plea is made for the publication of all segregation data (negative or otherwise), which can be collated biometrically at a later date.

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