A NEW EYE COLOR MUTANT IN THE MOUSE WITH ASYMMETRICAL EXPRESSION

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Most hereditary variations in the paired structures of animals with bi-lateral symmetry express themselves equally on both sides of the body. Exceptions to this rule are of interest as potential material for revealing new relationships in the mechanism of development. One such exception has been found recently in which an eye color mutation in the mouse fre-quently expresses itself differently in the right and left eyes.

The observations began while we were studying a case of *heterochromia iridis* which had been noted in a single animal in our stocks. This animal, a brown agouti pied female with a normal brown left eye and a much lighter rose- or ruby-colored right eye proved to be a somatic mosaic with no demonstrable change in the germ cells since we found no eye color variations in 6 F_1 and 31 F_2 offspring from this female. In searching for other possible occurrences of this type of variation we found that Dr. C. H. Danforth of Stanford University was maintaining a stock of silver mice with *heterochromia iridis*, a frequent condition being one eye ruby, the other.pink. Dr. Danforth kindly sent us several animals from this stock which we tested by crossing with our heterochromic female. The F_1 con-sisted of normal black-eyed animals only, indicating that the two occur-rences of heterochromia were not due to the same germinal change. In F_2, some animals with ruby eyes like those in the Danforth parent stock ap-peared, indicating segregation of a recessive ruby mutation.

We then turned our attention to the ruby mutation which had not been described previously. It proved to be a simple recessive to normal, out-crosses to wild type producing only wild type eye color in F_1 and 179 wild type (dark-eyed) and 54 ruby-eyed in F_2. The new eye color was easily scored at birth, the eyes of newborn mutants lacking the iris pigment ring of the wild type and resembling the unpigmented eyes of the mutants pink, pallid and albino.

The F_2 mutants obtained above contained the ruby gene (r) in combination with agouti and with black. Black agouti-ruby superficially resembles brown agouti since the black base of the fur is reduced to dark sepia while the yellow tip is only slightly reduced in intensity. Black-ruby is dull dark sepia or dark slate. Ruby obviously affects black hair pigments in the same direction but to a lesser extent than the mutants pink and pallid, while yellow which is unaffected by the latter mutants is somewhat reduced by ruby. Of the F_2 ruby animals nearly all showed ruby on both sides, but a few showed the heterochromia of the ruby grandparent.

These effects all resemble those of a mutation reported by Sô and Imai.[1] According to Grüneberg[2] (p. 42), who has had the Japanese text of this paper translated, the evidence shows that the ruby mutation of Sô and Imai was an allele of pink, the order of dominance being $P \gg p^r > p$. I have also examined a translation of this paper* and agree with Grüneberg that the evidence for allelism is conclusive. Consequently our ruby was tested by a pink-eyed agouti stock. F_1 was normal dark-eyed; F_2 consisted of 56 normal, 24 ruby-eyed and 28 pink-eyed. This is a sufficiently close approach to 9:3:4 ratio to suggest that our ruby and pink assort independently, the double recessive $pp\ rr$ being pink-eyed. A number of F_2 pink-eyed animals were tested. While most of them proved to be $pp\ Rr$, a few were $pp\ rr$. A double recessive stock of pink-eyed animals $pp\ rr$ was prepared of which the members were indistinguishable in eye color or coat color from the normal pink-eyed stock. Linkage relations of the new ruby with pink were tested by matings $\dfrac{PR}{pr} \times pp\ rr$ and $\dfrac{Pr}{pR} \times pp\ rr$. The former produced 36 dark-eyed (PR) and 87 light-eyed (pR, Pr and pr) young; the latter, 35 dark-eyed and 91 light-eyed. Combining these, the recombinations, 128, constituted almost exactly half of the 249 gametes tested, indicating free assortment of p and r.

Since two phenotypically indistinguishable but independent pink-eye mutations have been recorded in the mouse, it occurred to us that the Japanese investigators may have employed the p^2 (pallid) allele in their tests. We therefore tested ruby by p^2.† This produced only wild type offspring (38 observed) indicating that r is not a mutation at the p^2 locus. Similar tests by albino likewise gave no evidence of allelism of r and c^a. We may therefore assume that r marks a new locus in the mouse.

For reasons of interest in this laboratory we tested r for location in the T (Brachyury) chromosome. The tests produced the following results: $\dfrac{+T}{r+} \times ++$ gave 100 $++$, 114 $+\ T$, 124 $r+$, 100 $r\ T$. This fits an assumption of independent assortment ($p = 0.3$) and indicates that the r locus is probably not in the T chromosome.

Returning now to the asymmetrical expression of the new mutant we have noted that in the original stock as received from Professor Danforth, 53 animals carefully examined showed the following eye color distribution, as compared with rubies extracted from outcrosses to non-ruby stocks:

	BOTH EYES RUBY	LEFT EYE RUBY RIGHT EYE PINK	LEFT EYE PINK RIGHT EYE RUBY	BOTH EYES PINK
Original stock	22	11 + 5[a]	5 + 5[b]	5
Extracted ruby	50	4	3	1

[a] These were recorded as left eye dark ruby, right eye light ruby.
[b] These were recorded as right eye dark ruby, left eye light ruby.

While it is clear from these observations that asymmetry in eye color is frequent in the original stock (26 asymmetrical out of 53 carefully observed for this character), it is not evident that the asymmetry is due to the ruby gene, since after extraction from outcrosses the same ruby gene showed only about 12 per cent (7/57) asymmetry. The original stock was silver black pied (*aa BB sisi ss*). After outcrossing this to black agouti (*AA BB SiSi SS*), black and tan (*a^t a^t BB SiSi SS*) and pink-eyed black stocks (*aa BB pp SiSi SS*) the asymmetry of extracted ruby-eyed animals showed no relation to *A*, *a^t* or *a*, nor to the *Si-si* alleles. However out of 7 asymmetrical rubies, 5 were pied. Since the total number of pied animals observed in segregating generations was 9, we have proportions of asymmetrical ruby-eyed as 5/9 among pied and 2/48 among non-pied. The difference between these proportions is highly significant ($p = < 0.001$). It is probable therefore that the asymmetrical expression of ruby is greatly enhanced by pied spotting. This is to be taken as a preliminary indication only, since extended and systematic studies of other factors influencing asymmetrical expression have not been made.

In the present data there is some tendency for the left eye to be darker than the right, but the observations are too few to provide a test of this point. It is apparent that the expression of the ruby mutation depends upon an equilibrium which is influenced by both genetic and developmental factors which deserve further study.

The ruby mutation of Sô and Imai was similarly quite variable in density of eye color and this appeared to depend on other (unanalyzed) genetic factors. The Japanese authors also noted *heterochromia iridis* frequently in the ruby-eyed stock. They believed it to be genetically influenced but because of the weakness of such animals were unable to analyze it.

The mutation of Sô and Imai and that described here are the only two in mice in which heterochromia has been observed. This fact and their other similarities render them phenotypically almost identical yet they appear to be mutations at different loci. The only alternative to this is to assume

that the pink mutation with which Sô and Imai's ruby proved to be allelic was a third mutation, different from the p and p^2 of American and European stocks.   This is unlikely since the original pink mutation was introduced into Europe from Japan.

* Made by Toshi Miyazaki of Columbia University.

† Kindly supplied by Jackson Memorial Laboratory.

[1] Sô, M., and Imai, Y., "On the Inheritance of Ruby Eye in Mice," *Japanese Jour. Genet.*, 4, 8–9 (1926).

[2] Grüneberg, H., *The Genetics of the Mouse*, University Press, Cambridge, 1943, 412 pages.

ON NON-LINEAR PHENOMENON OF SELF-ROLLING

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Recently developed methods of non-linear mechanics offer interesting applications to a number of problems which have been known for a long time but which have remained unexplored so far.   A typical problem of. this kind is the so-called *self-rolling* of a ship provided with an antirolling stabilizing system.   It is well known that, if the phase of the control action is reversed, the ship, originally at rest in still water, begins to roll with gradually increasing roll angles until the amplitude reaches a stationary value after which the self-rolling continues indefinitely.   Although the physical nature of this phenomenon is sufficiently clear, it is impossible to determine the stationary amplitude from the linear theory for what eventually limits the amplitude is precisely the action of the various non-linearities which are generally left out in a simplified linear theory.

The differential equation of rolling in still water is

$$I\ddot{\theta} + a_1'\dot{\theta} + a_2'\dot{\theta}^2 + Dh(\theta) = 0 \qquad (1)$$

where θ is the angle of roll, D is the displacement of the ship, I is the virtual moment of inertia about the longitudinal axis through the center of gravity, a_1' and a_2' are the so-called Froude's coefficients of resistance to rolling (bilge keels, skin friction), and $h(\theta)$ is the lever arm of the restoring couple. For small angles of roll $h(\theta) \cong h_1 \theta$ where h_1 is the initial metacentric height assumed to be constant.   For larger angles one can approximate the quantity $Dh(\theta)$ by an expression of the form

$$Dh(\theta) = c_1'\theta - c_3'\theta^3 \qquad (2)$$