Notes

The Journal of Heredity 73:69–70. 1982.

Blonde, a new mutation in *Peromyscus maniculatus* affecting fur, skin, and eye pigmentation

Bruce M. Pratt and Robert J. Robbins

Abstract: An autosomal recessive mutation affecting hair and eye pigmentation was discovered in the F_2 progeny of wild-type deer mice, (*Peromyscus maniculatus*), trapped near East Lansing, Michigan. When homozygous, the mutation (designated as blonde, *bl*), reduces both black and yellow pigmentation deposited in the fur, reduces or eliminates pigmentation in the non-follicular melanocytes of the outer ear, peri-orbital skin and tail, slightly reduces the amount of pigmentation in the choroidal melanocytes, and completely eliminates pigmentation of the retinal epithelium.

In January 1976, in the *Peromyscus* colony maintained in the Biology Research Center of Michigan State University, two mutant mice were discovered in a litter produced from an inadvertent full-sib mating between the progeny of two unrelated wild-caught mice. Genetic tests have shown this mutation, designated as blonde (symbol *bl*) to be an autosomal recessive.

Materials and Methods

During the course of this study, the blonde mutation was maintained by alternate generations of *bl*/+ × *bl*/+ and *bl*/*bl* × +/+ matings. Hairs from the mid-dorsal area were either dry mounted or cleared and mounted according to Russell[12]. Eyes from +/+ and *bl*/*bl* mice were fixed in Bouin's fluid, prepared for normal paraffin histology, sectioned at 15 μm and stained with Harris's hematoxylin and eosin.

Results

Table I summarizes a series of matings involving blonde mice and indicates that blonde is an autosomal recessive mutation with normal viability. Matings between blonde mice always produced only blonde progeny.

The agouti pigmentation pattern of the dorsal and lateral fur of the wild-type mouse consists of a subterminal band of yellow pigmentation and a black pigmented tip of the distal segments of the zig zag and auchene hair types. The agouti pigmentation pattern of the ventral fur of the wild-type mouse consists of a black base and unpigmented tip in all of the hair types. The wild-type mouse also has a ring of intensely dark pigmented skin around the eyes, dark hair and skin on the dorsal tail, and darkly pigmented hair and skin on the outer margins of the ears. Microscopically, the nonfollicular melanocytes of the ear are found predominantly within the dermis, with relatively few melanocytes in the epidermis.

Blonde affects the normal agouti-hair pigment-distribution in two ways: 1) the proximal regions of the hairs contain slightly less black pigmentation, and 2) the tips of the agouti hairs in the dorsal and lateral fur do not have any black pigmentation. These two effects combine to give the mutant mice a noticeably lighter fur coloration. Microscopically, the dorsal agouti hairs of the blonde mice contain slightly fewer eumelanosomes than normal in the proximal regions of the hair. The subterminal bands contain considerably reduced numbers of phaeomelanosomes. The tips of the hairs contain either very few or no eumelanosomes in either the cortex or medulla.

Table I. Segregation of the blonde character in deer mice

Matings male × female	Progeny *bl*	Progeny +	Total	χ^2
+/+ × *bl*/*bl*	0	70	70	
bl/*bl* × +/+	0	24	24	
bl/+ × *bl*/+	82	208	290	1.660
				$P > 0.10$

Blonde affects skin and fur pigmentation in several ways: the skin around the eyes is unpigmented; the dorsal tail has white to light gray hair and skin; and the ears of the blonde mice have pale yellow to white hair and no visible melanotic skin pigmentation. Microscopically, the hair on the ear contains only a few melanosomes, and the ear skin contains no non-follicular melanocytes.

The homozygous blonde mice can be recognized at birth by a lack of visible pigmentation in the eye, causing a superficial resemblance to albino mice. As the mice mature gradual pigmentation occurs so that the eyes of adult blonde mice are dark ruby in color. Microscopically, the choroidal pigmentation of the adult blonde eyes is slightly reduced in thickness. More striking is the complete absence of pigmentation in all regions of the retinal epithelium of the blonde mice (see Figure 1). At the light microscopic level the neural layer of the retina appears normal.

Discussion

The effects of the blonde mutation on the cutaneous pigmentation of the mice (i.e., an increased prominence of the subterminal yellow band of the hair) appear similar to those described for a dominant mutation in *Peromyscus*, wide-band (*Nb*)[9]. However, the

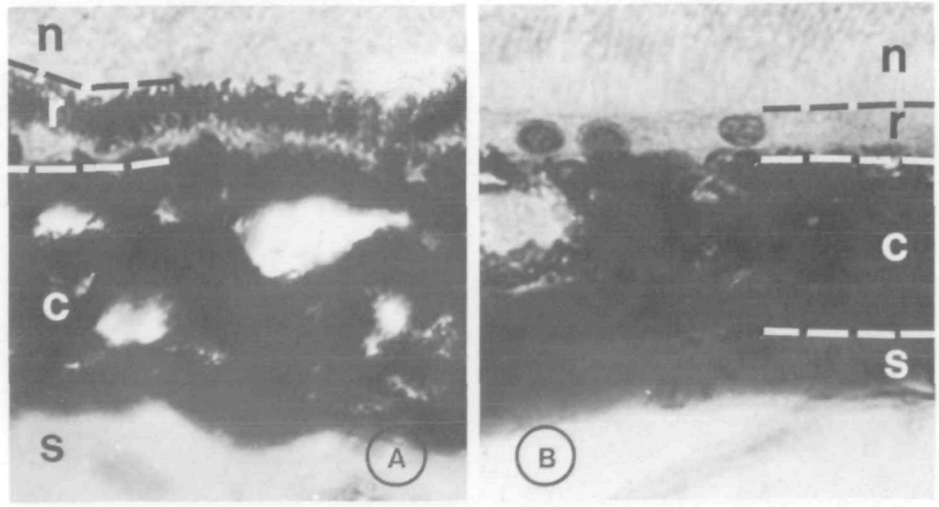

FIGURE 1 Light micrographs of retinal epithelium and choroid of the eye (n = neural retina, r = retinal epithelium, c = choroidal melanocytes, s = sclera): *A*—wild-type eye (1060 ×). *B*—*bl*/*bl* eye (1060 ×); note the reduced thickness of the choroidal pigmentation and the lack of pigmentation in the retinal epithelium of the *bl*/*bl* eye.

Dr. Pratt obtained his Ph.D. with the Department of Zoology at Michigan State University and is currently a postdoctoral fellow in the Biology Department, Yale University, New Haven CT 06511. Dr. Robbins is assistant professor in the Department of Zoology, Michigan State University, East Lansing Michigan 48824. The authors express their appreciation to Ms. Kathleen McGleish, whose alertness in discovering the original mutants among mice scheduled for disposal permitted this study; and to Dr. C. L. Markert, Biology Department, Yale University, for providing a critical review of the manuscript. Reprint requests should be addressed to the senior author. At the time of writing, stocks of blonde mice are being maintained by C. L. Markert, Yale University; D. A. Dewsbury, Department of Psychology, University of Florida, Gainesville, FL 32611; S. Van Ooteghem, Department of Biology, University of Michigan, Ann Arbor, MI 48104; and R. J. Robbins, Department of Zoology, Michigan State University.

differential effects of blonde on the pigmentation of the retinal epithelium and choroidal melanocytes appears to distinguish the blonde mutation from the wide-band mutation. Likewise, the effects of the blonde mutation on the eye seem somewhat similar to those described for the mutation red eye[3]. However, unlike red eye, blonde does not cause heterochromia and does affect hair pigmentation.

In the house mouse, the mutation pallid (*pd*) has similar phenotypic effects: an absence of melanosomes in the retinal epithelium[15], reduced melanization of the choroidal melanocytes[2], and reduced phaeomelanin and eumelanin in the hair[10,11]. Pallid, considered to be a genetic manganese deficiency[1,13] also causes a partial or complete absence of otoliths, resulting in behavioral and postural defects[4,5]. The penetrance of the pallid-induced otolith defect is influenced by genetic background[5]. Since comparable postural defects were not noted in the blonde mice, it seems probable that the blonde mutation, on its present genetic background, does not radically affect otolith development.

Insights into the developmental regulation of pigmentation can be gained by comparing the phenotypic effects of different mutations that affect occulocutaneous pigmentation. Such mutations can be classified into three major categories: 1) mutations with similar effects on both neural crest-derived and retinal epithelial melanocytes; 2) mutations affecting primarily, or exclusively, the neural crest-derived melanocytes; and 3) mutations affecting primarily, or exclusively, the optic cup-derived retinal epithelial melanocytes[6]. Mutations of the first type, e.g., albino, brown, dilute, pink eye, act on those steps of melanosome synthesis and melanogenesis that are common to both types of melanocytes[14]. Mutations of the second type, which have been critically examined (e.g., dominant spotting and steel in the house mouse[7,8]) act by blocking some step(s) of neural crest melanocyte differentiation before the onset of melanosome synthesis and melanogenesis. Mutations of the third type (e.g., blonde in *Peromyscus* and pallid in the house mouse) quantitatively decrease pigmentation in the choroidal melanocytes and qualitatively block melanosome synthesis and/or melanogenesis in the retinal epithelium. This differential phenotypic effect on the two developmentally distinct classes of melanocytes demonstrates the existence of developmental regulatory events of melanosome synthesis and/or melanogenesis that are restricted to the retinal pigment epithelium and are not common to both the neural crest- and optic cup-derived melanocytes.

References

1. ERWAY, L. C., A. S. FRASER, and L. S. HURLEY. Prevention of congenital otolith defect in pallid mutant mice by manganese supplementation. *Genetics* 67:97–108. 1971.
2. HEARING, V. J., P. PHILLIPS, and M. A. LUTZNER. The fine structure of melanogenesis in coat color mutants of the mouse. *J. Ultrastruct. Res.* 43:88–106. 1973.
3. HUESTIS, R. R. and R. WILLOUGHBY. Heterochromia in *Peromyscus*. *J. Hered.* 41:287–290. 1950.
4. LYON, M. F. Hereditary absence of otoliths in the house mouse. *J. Physiol.* 114:410–418. 1951.
5. ———. Absence of otoliths in the mouse: An effect of the pallid mutation. *J. Genet.* 51:638–650. 1953.
6. MARKERT, C. L. and W. K. SILVERS. The effects of genotype and cell environment on melanoblast differentiation in the house mouse. *Genetics* 41:429–450. 1956.
7. MAYER, T. C. A comparison of pigment cell development in albino, steel, and dominant spotting mutant mouse embryos. *Develop. Biol.* 23:297–309. 1970.
8. ——— and M. C. GREEN. An experimental analysis of the pigment defect caused by mutations at the *W* and *Sl* loci in mice. *Develop. Biol.* 18:62–75. 1968.
9. MCINTOSH, W. B. Linkage in *Peromyscus* and sequential tests for independent assortment. *Contrib. Lab. Vertebr. Biol. Univ. Mich.* 73:1–27. 1956.
10. ROBERTS, E. A new mutation in the house mouse (*Mus musculus*). *Science* 74:569. 1931.
11. ——— and J. H. QUISENBERRY. Linkage of the genes for nonyellow (y) and pink-eye (p_2) in the house mouse (*Mus musculus*) *Am. Nat.* 69: 181–183. 1935.
12. RUSSELL, E. S. A quantitative histological study of the pigment found in the coat-color mutants of the house mouse. I. Variable attributes of the pigment granules. *Genetics* 31:327–346. 1946.
13. SHRADER, R. E., L. E. ERWAY, and L. S. HURLEY. Mucopolysaccharide synthesis in the developing inner ear of manganese-deficient and pallid mutant mice. *Teratology* 8:257–266. 1973.
14. SILVERS, W. K. The Coat Colors of Mice. Springer-Verlag, NY. 1979.
15. THERIAULT, L. L. and L. S. HURLEY. Ultrastructure of developing melanosomes in C57 black and pallid mice. *Develop. Biol.* 23:261–275. 1970.

The Journal of Heredity 73:70. 1982.

A Fortran computer program for calculating linkage intensities from F_2 data

David K. Shortess

ABSTRACT: A Fortran computer program is presented that calculates map distances from F_2 data that may involve gene interaction between one or both of the linked genes and other genes. Probable errors based on the sample sizes also are calculated.

ALTHOUGH testcross data are always the most reliable for the calculation of linkage intensities, these data are not always available. Frequently F_2 data must be used. When the linked genes involved are expressed simply, with no gene interaction, the calculations are fairly straight-forward, but if complex expressions are involved, the calculations become excessive. Several methods have been devised to overcome this problem. One of the most popular over the years has been the product method of Immer[1]. Although his formulas and tables were published over 50 years ago, a review of the literature[2] indicates they are still being used.

The tables that Immer derived from his formulas are fairly easy to use, but they often are not readily available. I have, therefore, developed a Fortran computer program that uses those formulas to calculate map distances from complex F_2 data. The language used in this program is Fortran IV and is designed for televideo data input. In cases where the expression of one or both traits involved are controlled by more than one allelic pair, it is assumed that only one gene in each expression is involved in the linkage.

When the program is executed, there is a brief introductory statement, after which the F_2 data are requested. These data must be entered in the correct order, with the value of the double dominant phenotype given first, followed by the two single dominant, single recessive phenotypes, with the value of the double recessive phenotype last. The program then prompts for a choice of individual phenotypic ratio combination, such as 3:1 and 3:1, or 9:7 and 3:1. Upon entering that choice, the program will calculate the map distance based on the data, as well as the probable error, based on the sample size. It is not necessary to indicate whether the genes in the F_1 were in coupling or repulsion phase. The program will determine that automatically. The results will be displayed on the video terminal screen as "Map units = xx.x +/−yy.y", where xx.x is equal to the map units, and yy.y is equal to the probable error. Since Fortran does not have the provision for displaying the conventional ±, the symbol +/− is used. The program then asks if there is another test to be run.

I have found this program to be useful not only as a research tool, but as an instructional aid as well. I will be happy to provide a copy of the program to anyone who desires it.

The author is associate professor of biology, New Mexico Institute of Mining and Technology, Socorro, NM 87801.

References

1. IMMER, F. R. Formulae and tables for calculating linkage intensities. *Genetics* 15:81–98. 1930.
2. SCIENCE CITATION INDEX. Institute for Scientific Information, Philadelphia, PA. 1975–1980.