Coat Color Genetics of *Peromyscus*: IV. Variable White, a New Dominant Mutation in the Deer Mouse

K. Cowling, R. J. Robbins, G. R. Haigh, S. K. Teed, and W. D. Dawson

The variable white mutation arose spontaneously in 1983 within a laboratory stock of wild-type deer mice (*Peromyscus maniculatus*). The original mutant animal was born to a wild-type pair that had previously produced several entirely wild-type litters. Other variable white animals were bred from the initial individual. Variable white deer mice exhibit extensive areas of white on the head, sides, and tail. Usually a portion of pigmented pelage occurs dorsally and on the shoulders, but the extent of white varies from nearly all white to patches of white on the muzzle, tip of tail, and sides. The pattern is irregular, but not entirely asymmetrical. Eyes are pigmented, but histologically reveal a decrease in thickness and pigmentation of the choroid layer. Many variable white animals do not respond to auditory stimuli, an effect that is particularly evident in animals in which the head is entirely white. Ataxic behavior is also prevalent. Pigment distribution, together with auditory and retinal deficiencies, suggests a neural crest cell migration defect. Breeding data are consistent with an autosomal semidominant, lethal mode of inheritance. The trait differs from two somewhat similar variants in *Peromyscus*: from dominant spot (*S*) in extent and pattern of pigmentation and from whiteside (*ws*), an autosomal recessive trait, in the mode of inheritance and viability. Evidence for possible homology with the *Va* (varitint-waddler) locus in house mouse (*Mus*) is presented. The symbol *Vw* is tentatively assigned for the variable white locus in *Peromyscus*.

The first variable white mutant animal appeared spontaneously in 1983 in a long-established stock of wild-type prairie deer mice, *Peromyscus maniculatus bairdii*, maintained at Michigan State University. A mated pair that had previously yielded more than 50 wild-type progeny produced a single female exhibiting the variable white phenotype. This appears to be the first reported case in *Peromyscus* that clearly represents a new laboratory mutation. Other genetic variants described in the genus appeared in wild-captured animals or among early-generation progeny of wild-caught animals where the mutant allele likely was present in the ancestral natural population.

The initial mutant female was crossed to a wild-type male and produced variable white and wild-type offspring of both sexes. The phenotype and mode of inheritance was analyzed in this and subsequent generations.

Description

Although the original mutant animal was predominantly white, considerable variation occurs among individuals in the extent of unpigmented pelage (Figure 1). There is extensive white on the face, ears, and forehead. The tail is largely unpigmented in most individuals and is always white at the tip. In unpigmented areas the individual hairs are white throughout. The mid-dorsum is nearly always pigmented, but not clearly delineated. The margins of the pigmented areas appear ragged and uneven, and the pigment is diluted, giving pigmented regions a grayish-tan wash. The eyes are dark like those of wild-type deer mice.

Microscopic analysis of fixed, paraffin embedded, stained sections of skin revealed an absence of unpigmented melanocytes (clear cells) in hair follicles from the white areas of the skin, while pigmented melanocytes were present in the pigmented portions of the pelage. Sections of the choroid layer of the retina, embedded in plastic to prevent deformation and visualized with Lee's stain, exhibited decreased pigmentation and were thinner than in wild-type mice (Figure 2).

A noticeable feature of the mutant phenotype is variability in response to auditory stimuli. Some individuals appear to be deaf, while others react normally (Table 1). Auditory deficiency was more pronounced in males than in females. In general, animals with extensive white on the head were more likely to exhibit deafness, indicating a basic defect in neural crest cell migration, the common origin of both melanocytes and inner ear sensory cells. An analogous situation occurs with *Va*- and some *W*-locus mutants in house mouse (*Mus*) (Doel 1954, 1970). Most variable white *Peromyscus* display degrees of ataxic or circling behavior. Some have difficulty righting themselves when overturned, stumble when walking, and swim with difficulty. Running and swimming in tight circles is characteristic of some animals in which the phenotype is highly expressed.

Hematocrits of two wild-type *P. m. bairdii* (+/+), five heterozygous variable white (*Vw*/+), and four dominant spot (*S*/+) adults ranged from 0.42 in one of the wild-type to 0.51 in one of the variable white animals. No hematocrits deviated significantly from the overall mean (0.46 ± 0.05), which is a normal value. Thus, there was no evidence in either heterozygous mutant for erythrocytic deficiency analogous to anemia seen in some combinations of *W* mutants in *Mus*.

Inheritance

We analyzed inheritance of variable white from 10 intercrosses and 48 backcrosses. Progeny were weaned and phenotypes were scored at 25–30 days of age. Five litters from three of the intercross matings were wet-nursed on unrelated deer mice at birth. A total of 1,018 weaned progeny were scored for sex and coat color phenotype. Pre-weaning losses were recorded for most of the litters from which at least one young was weaned.

Transmission of the trait suggested a monogenic autosomal dominance inheritance mode, but reduced viability and possible sterility also seemed to be associated with the condition. Therefore, genetic segregation data were tested against two hypotheses: complete dominance and semidominance with lethality. Overall, the F_2 exhibited a closer fit to the 2:1 ratio expected if the homozygote was an embryonic or early postnatal lethal than the 3:1 ratio expected for complete dominance (Table 2). The backcrosses, of heterozygous (*Vw*/+) females to wild-type (+/+) males produced more wild-type offspring than expected, but deviation in the reciprocal backcross was trivial. When reciprocal backcrosses were combined, departure from expected was not significant. There was no evidence of sex-linked inheritance, but a deficiency of female variable white segregants was apparent in the first backcross.

Litter size at birth was compared with litter size at weaning for a subset of litters in which at least one young was weaned (Table 3). Postnatal pre-weaning mortality ranged from 11% to 20% and represented an average loss of less than one animal per litter. This is insufficient to account completely for the deficiency of variable white animals in the F_2. Litter size at both birth and weaning is larger by nearly one among the +/+ × *Vw*/+ backcross progeny than in the other two crosses, indicating greater prenatal mortality or reduced female fertility among *Vw*/+ mothers. When total pre-weaning mortality of all F_2 litters born was considered, including that from matings where none

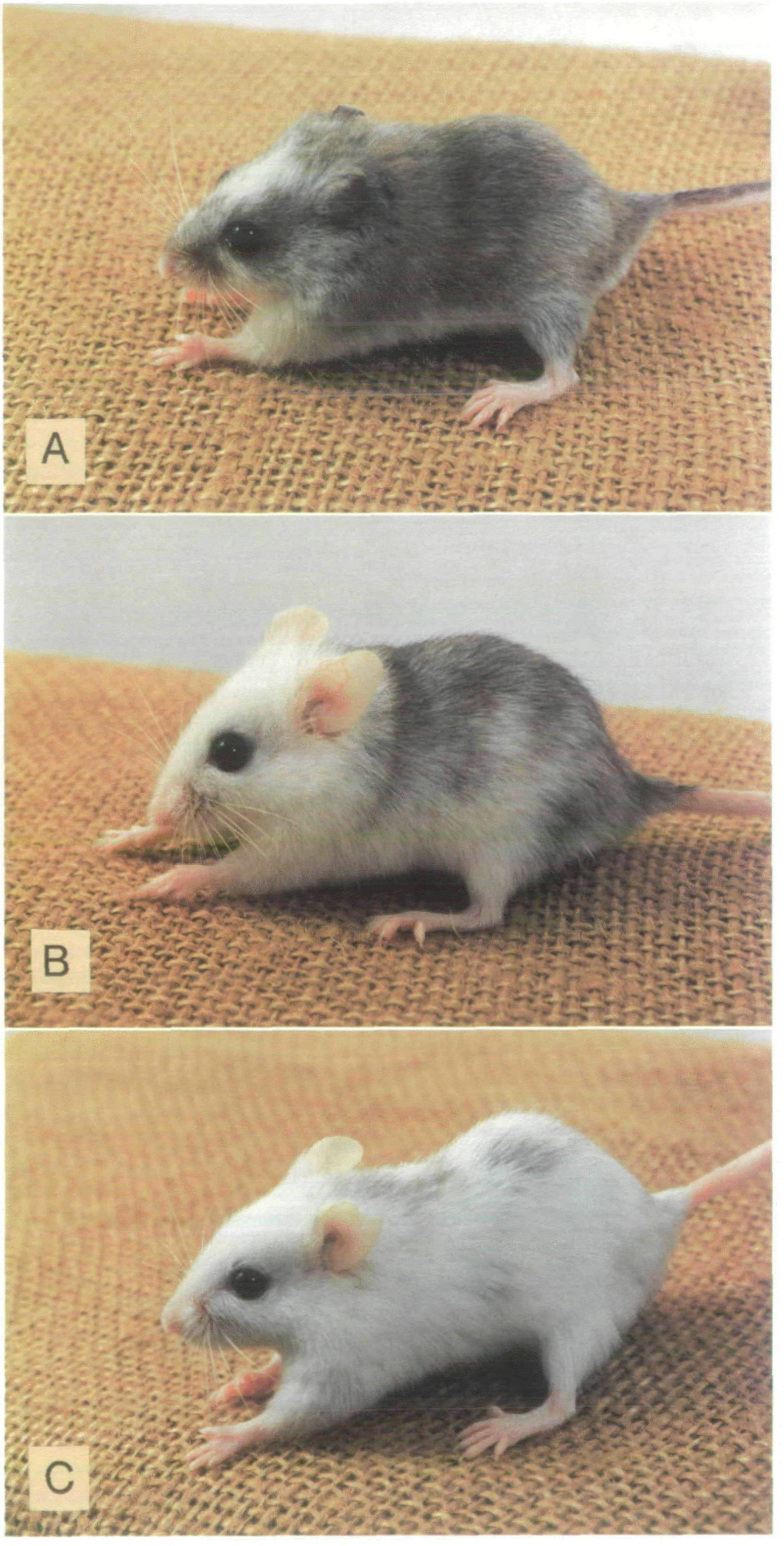

Figure 1. Variation in expression of the variable white phenotype: (**A**) minimum expression; (**B**) typical expression; (**C**) maximum expression.

Table 1. Response[a] of 40 test animals to auditory stimuli

	Wild-type females	Wild-type males	Variable white females	Variable white males
	+ + +	+ +	−	−
	+ + +	+ + +	−	+
	+ + +	+ + +	+	−
	+ +	+ +	+ +	−
	+ + +	+ + +	−	−
	+ + +	+	−	−
	+ + +	+ +	−	−
	+ + +	+ +	+ + +	−
	+ +	+ +	+ + +	−
	+	+ +	+ + +	−
Mean	2.6	2.2	1.2	0.1
	Wild type: 2.4		Var. white: 0.65	

[a] Key: − no response (scaled 0); + minimal response (1); + + ears flattened (2), + + + ears flattened and body flexed (3).

were weaned, the losses were 34.0%, which is greater than neonatal mortality in matings between wild-type individuals of this species.

To determine whether the pre-weaning losses involved principally variable white individuals, we established three matings between heterozygotes and regularly observed progeny from birth, scoring phenotype as early as possible. Most of these litters were lost before scoring could be accomplished, but both white and wild-type individuals occurred among the small number surviving. In five litters from these same matings, which were wet-nursed from birth on unrelated +/+ females, nine variable white and seven wild-type progeny survived to weaning and five were lost before weaning. Even if the five lost were all variable white, there would be a deficiency of variable white in these litters. The collective data indicate that both prenatal and early postnatal mortality among variable white progeny is common.

A sex bias was noted in one backcross. When female *Vw*/+ were backcrossed to wild-type males, there was a deficiency of variable white females among the progeny. In this backcross 19.7% of the progeny were lost between birth and weaning, based on those litters in which at least one young was weaned, or 28.8% based on all litters born. The reciprocal backcross segregated normally, with only 10.6% pre-weaning mortality, which is typical for *P. maniculatus* wild-type stocks (mean = 8.1% ± 0.1%). Variable white mothers showed poorer success in rearing their variable white progeny than did their wild-type counterparts.

We tested the interaction between variable white (*Vw*) and the dominant spot

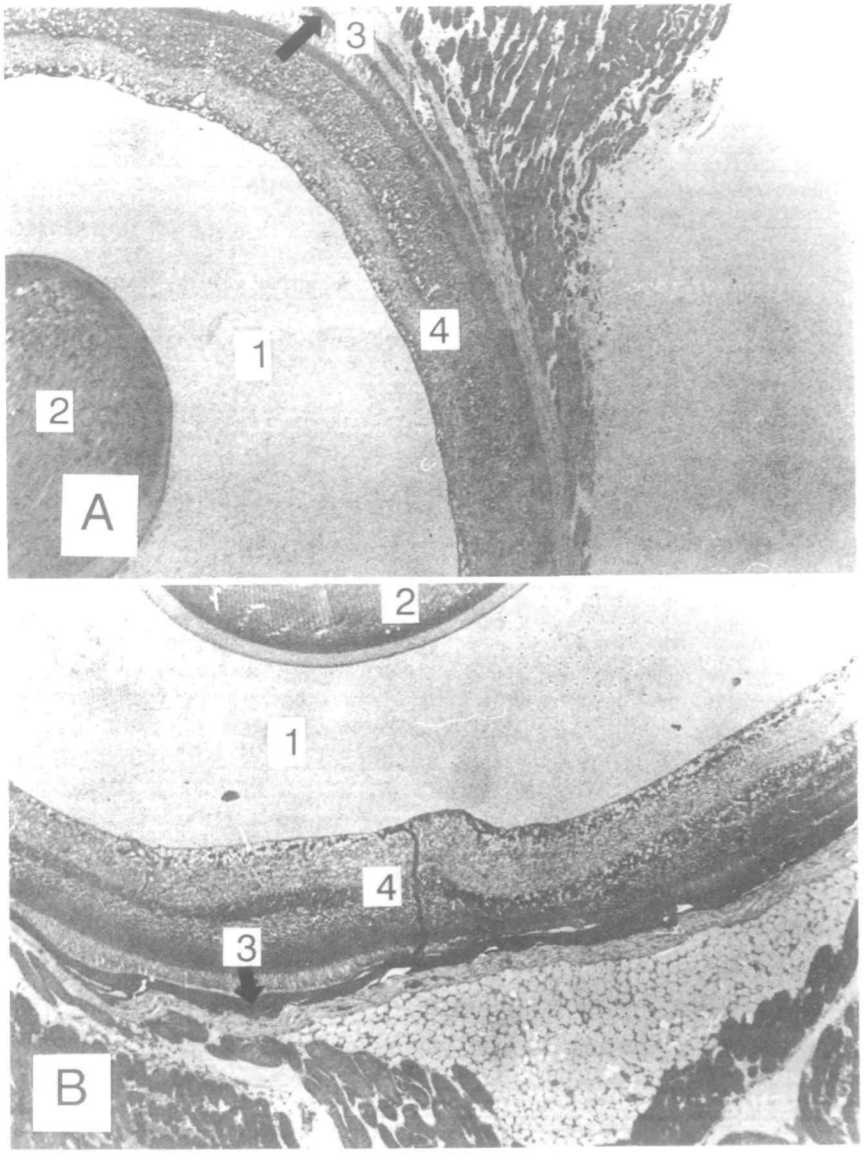

Figure 2. Effect of the variable white gene on the choroid layer of the eye. Sections through the eye (plastic imbedded, Lee's stain, 40×): (**A**) variable white mutant; (**B**) wild type Key: 1 = vitreous humor; 2 = lens, 3 = choroid, 4 = retina. Note the decreased thickness of the choroid layer in variable white compared with the wild type.

(*S*) mutation. Three matings of *Vw*/+ females to *S*/+ produced 58 weaned offspring, and one reciprocal mating yielded 11 progeny. Of the 69 total offspring, 49 were variable white and/or spotted and 20 were scored as wild type. Although these mutant phenotypes could not be scored unambiguously, 25 progeny appeared variable white and 24 were spotted. When mutant phenotypes are pooled, the results are consistent with the 3:1 ratio ($\chi^2 = 0.585$, $.50 > P > .30$) expected if both genes are dominant whether the genes are allelic or not. Most progeny showed an intermediate state of spotting, suggesting a lack of interaction between the *S* and *Vw* factors. An additive effect of the two dominants, whether allelic or representing separate loci, would likely produce virtually all-white animals, an effect that was not observed.

The mean size of the 27 weaned litters in the *Vw*/+ × *S*/+ cross was 2.55, compared with 4.6 in 40 *S*/+ × +/+ litters and 3.55 in 40 *Vw*/+ × +/+ litters. These data indicate possible prenatal lethal interaction between the dominant factors.

Since variable white in *Peromyscus* produced phenotypic features similar to those produced by varitint-waddler (*Va*) and white (*W*) mutations in *Mus*, we performed a linkage test to determine if *Vw* maps to a conserved chromosomal region. In the mouse the *Va* locus is closely linked (4 cM) to the alcohol dehydrogenase locus (*Adh-1*) on chromosome 3, whereas the *W* locus is linked (9 cM) to the albumin locus (*Alb-1*) on chromosome 5. Since the interfertile species *P. maniculatus* and *P. polionotus* differ for variants at both the *Adh-1* and *Alb-1* loci (Dawson 1982), an interspecific backcross linkage test was performed. Female variable white heterozygous (*Vw*/+) *P. maniculatus*, known to be homozygous for the "fast" albumin electromorph and *Adh-1* restriction fragment length pattern (RFLP) 1, were crossed to non-*Vw P. polionotus* which breed true for a "slow" albumin and *Adh-1* RFLP 2. Variable white F_1 offspring, which were heterozygous at all three loci, were then backcrossed to wild-type *P. polionotus*, which were homozygous for the three genes. We scored 10 backcross progeny for coat color, serum albumin starch gel electrophoretic variants, and alcohol dehydrogenase RFLPs. RFLPs were identified by Southern blot hybridization of tail clip DNA digested with *PvuII* and *PstI* to a deer mouse ADH probe kindly provided by M. R. Felder. Six additional progeny were scored for only coat color and albumin type. Recombination of variable white with these test markers is shown in Table 4. The sample size is sufficient to detect close linkage of *Vw* to either of the test loci if the recombinant fractions correspond to those in *Mus*. No evidence of close linkage of *Vw* to either the *Alb-1* or *Adh-1* locus in *Peromyscus* was obtained.

Discussion

Variable white is the fourth dominant or semidominant monogenic coat color mutation reported for the deer mouse of 20 color and pattern mutations recorded in the genetic literature. Of these four dominants, three (dominant spot, whitecheek, and variable white) involve extension of white into normally pigmented regions of the pelage. Additionally, at least five pattern modifiers that increase the amount of white in the coat of wild oldfield mice (*P polionotus*) are inherited as dominant or incompletely dominant traits (Bowen and Dawson 1977). One recessive mutation, whiteside, in the deer mouse also extends white areas of the coat.

Dominant spot (*S*), originally called "whiteface," produces a white spot on the forehead, a white tip on the tail, and all-white hair areas on the ventrum (Feldman 1936). Expression varies, and in some animals the head is entirely white. By the addition of minor recessive spotting factors to the background, combined with selection for maximum expression, Feldman

Table 2. Inheritance of the variable white mutation in deer mice, assuming autosomal dominance; in square brackets is the test of the alternate hypothesis, assuming semidominance with homozygous prenatal and/or early postnatal lethality

Mating type			Phenotype					Analysis[a]				
				Variable white		Wild type						
Female	Male	No. of matings		Female	Male	Female	Male	Unit	Segregation	Sex	Interaction	Total
Vw/+	× *Vw*/+	10	observed	43	52	24	23	Ratio	3:1 [2:1]	1:1	1:1	3:3:1:1 [2:2:1:1]
								χ^2	4.545* [.001]	0.345	0.570	5.756 [.880]
			expected	53.25	53.25	17.75	17.75	df	1	1	1	3
				[47.33]	[47.33]	[23.66]	[23.66]	*P*	.05–.025 [.99–.975]	.6–.5	.5–.4	.2–.1 [.9–.8]
Vw/+	× +/+	14	observed	50	71	72	88	Ratio	1:1	1:1	1:1	1:1:1:1
								χ^2	5.139*	4.612*	0.057	10.374*
			expected	70.25	70.25	70.25	70.25	df	1	1	1	3
								P	.025–.01	.05–.025	.9–.8	.025–.01
+/+	× *Vw*/+	34	observed	145	144	165	141	Ratio	1:1	1:1	1:1	1:1:1:1
								χ^2	0.430	0.968	0.813	2.425
			expected	148.75	148.75	148.75	148.75	df	1	1	1	3
								P	.6–.5	.4–.3	.4–.3	.5–.4
Backcrosses combined		48	observed	195	215	237	229	Ratio	1:0	1:1	1:1	1:1:1:1
								χ^2	3.453	0.138	0.832	4.639
			expected	219	219	219	219	df	1	1	1	3
								P	.1–.05	.8–.7	.4–.3	.3–.2

* Significance at $P < .05$.

[a] Yates' correction, as applicable.

produced animals with extensive white on the head, body, and tail, such that they were essentially white with black eyes. Dominant spot differs from variable white in that the spotting is more discrete in the former (Figure 3), is less extensively expressed on the head and body, and tends to be more bilaterally symmetrical in expression.

Whiteside (*ws*) is similar in phenotype to variable white, but is inherited as a recessive character (McIntosh 1956). The animals do not respond to auditory stimuli. This mutation is now extinct in laboratory stocks.

The whitecheek (*Wc*) trait occurs in natural populations of *P. polionotus* along the Florida coasts. In interspecific hybrid crosses with *P. maniculatus*, it is inherited as a dominant (Blair 1944). The pigmentation of the cheek below the eye, typical of most *Peromyscus*, is absent. This gene may be identical to one of the four face pattern genes known in *P. polionotus* (Bowen and Dawson 1977). The subspecies *P. p. leucocephalus* (Santa Rosa Island beach mouse) from the Gulf Coast of Florida has extremely pale color due to the action of genes at eight to 11 loci, most of which are dominantly inherited (Bowen and Dawson 1977; Sumner 1930). The pattern of this subspecies superficially resembles the phenotype of some variable white *P. maniculatus*. However, beach mice are not deaf.

The phenotypes of variable white and dominant spot in the deer mouse resemble certain phenotypes produced by *Va* and *W* alleles in laboratory mice (Silvers 1979). In each instance white spotting is inherited dominantly, there is evidence of neural crest cell involvement, and the possibility of homozygote lethality or sterility exists. In both species expression of spotting can be increased by independent minor modifiers. Phenotypically the dominant spot trait is most like W-fertile (W^f) in *Mus* (Guenet et al. 1979), and variable white in *Peromyscus* resembles varitint-waddler (*Va*) (Cloudman and Bunker 1945) and W-viable (W^v) (Markert and Silvers 1956). However, neither of the *Peromyscus* mutants is anemic, as is the case in some W-allele combinations in *Mus* (Russell 1949). The ataxia seen in variable white *Peromyscus* is like that in varitint-waddler *Mus*. Furthermore, details of the coat color phenotype, particularly the "faded" dorsal and lateral patches which break up the white areas, are more characteristic of varitint than white. *Va* also appears to be semidominant, although no individual variable white *Peromyscus* corresponded to the nearly completely white homozygote (*Va*/*Va*) recorded by Cloudman and Bunker (1945). We suspect that few, if any *Vw*/*Vw* homozygotes in *Peromyscus* survive to weaning.

Our recombination data showed that *Vw* does not map near either the *Adh-1* or the *Alb-1* locus as do *Va* and *W*, respectively, in *Mus*. At most, the variable white locus is only loosely linked to *Alb-1*, and, apparently, assorts independently of *Adh-1*. If linkage homology is conserved between

Table 3. Comparison of litter sizes at birth and at weaning among variable white mating classes (data based on litters from which at least one was weaned)

Mating class (female × male)	No. of litters	Litter size[a]		Difference	Loss (%)
		At birth	At weaning		
Vw/+ × *Vw*/+	39	4.103 ± 0.355	3.615 ± 0.352	0.488	11.9
Vw/+ × +/+	70	4.129 ± 0.299	3.314 ± 0.285	0.815	19.7
+/+ × *Vw*/+	96	5.094 ± 0.364*	4.552 ± 0.279*	0.542	10.6

[a] Mean ± 2X the standard error.

* Differs significantly from other two classes.

Table 4. A test for close linkage of variable white (*Vw*) with alcohol dehydrogenase-1 (*Adh-1*) or albumin (*Alb-1*) in *Peromyscus*

Backcross progeny genotypes		Observed	Expected if independent	Expected if linked[a]
Vw/+	Adh^1/Adh^2	4	2.5	4.8
Vw/+	Adh^2/Adh^2	3	2.5	0.2
+/+	Adh^1/Adh^2	2	2.5	0.2
+/+	Adh^2/Adh^2	1	2.5	4.8
Recombinants		5	5	0.4
Vw/+	Alb^1/Alb^a	9	4	7.28
Vw/+	Alb^a/Alb^a	3	4	0.72
+/+	Alb^1/Alb^a	1	4	0.72
+/+	Alb^a/Alb^a	3	4	7.28
Recombinants		4	8	1.44

[a] Assuming linkage is same as *Va* to *Adh-1* and *W* to *Alb-1* in *Mus*.

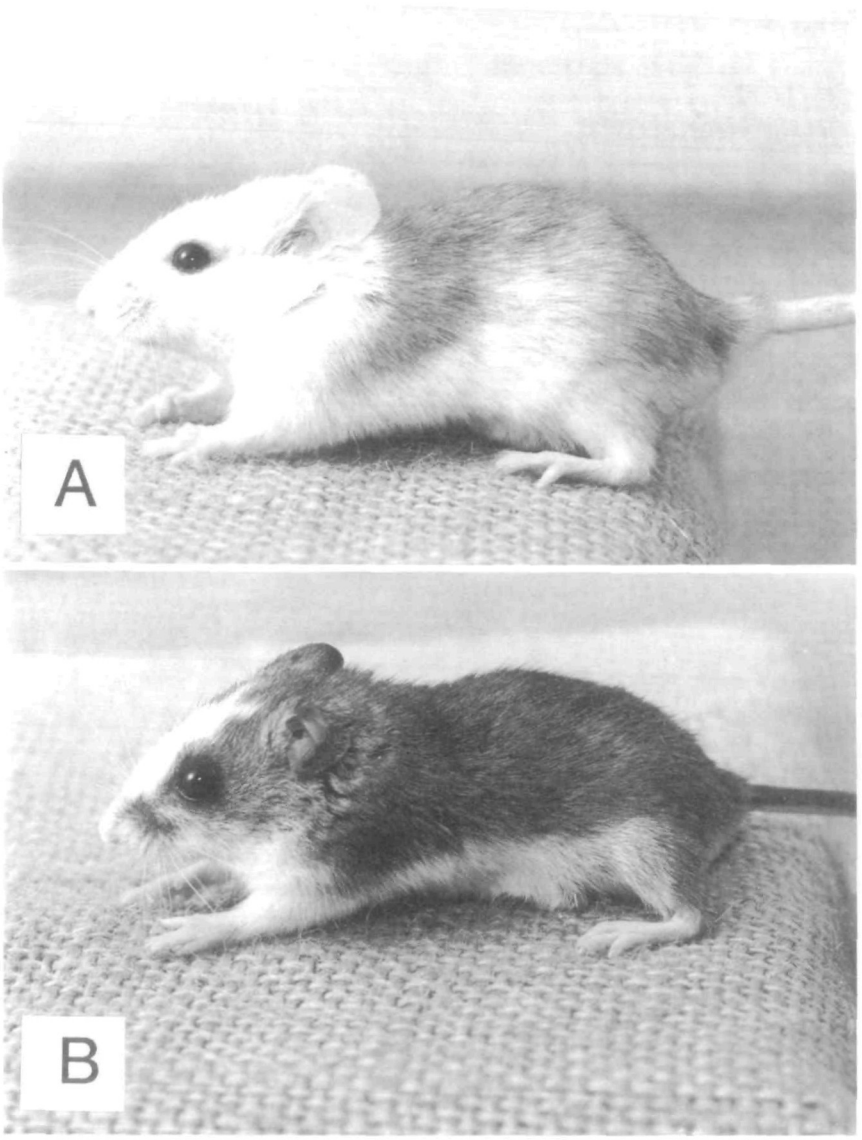

Figure 3. Comparison of (**A**) variable white (*Vw*/+) with (**B**) dominant spot (*S*/+).

Mus and *Peromyscus* for the loci of interest, then variable white may represent a third locus which has not been identified in *Mus*. Alternatively, *Vw* may be the homologue of *Va*, or possibly *W*, but has been separated from *Adh-1* (or *Alb-1*) by a chromosomal rearrangement since phylogenetic divergence of *Mus* and *Peromyscus* ancestry about 20 million years ago. Because of similarity in details of coat color, behavior, and genetic segregation, varitint-waddler is a better candidate for a claim of homology. However, since linkage data did not support this conclusion, we recommend a tentative designation of the variable white locus in *Peromyscus* as *Vw*, and propose that the symbol *S*, for dominant spot, be retained for the present. This nomenclature may be revised as the genetics of these traits become better understood.

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Genetic Impact of an Unusual Group Mortality Among Humpback Whales

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Mass mortalities, due to infectious disease or toxic algal blooms, are known to have severe demographic impacts on marine mammal populations. The genetic impacts of these events, however, have received little attention. To investigate the genetic consequences of an unusual group mortality among humpback whales, we compared the mitochondrial DNA haplotypes of 10 whales poisoned by mackerel contaminated with a dinoflagellate neurotoxin to those of 32 live whales from the same regional population. Two haplotypes that were rare in the reference sample of live whales accounted for eight of the 10 poisoned whales. A randomized test of independence, based on 500 permutations of the data matrix, showed significant differences in the frequencies of haplotypes in the two samples ($P < .002$).