THE INHERITANCE OF SEVEN ERYTHROCYTE ANTIGENS IN THE DOMESTIC MINK¹

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NUMEROUS blood group studies in many species of domestic animals have been made during the past 20 years. SAISON (1962) appears to be the first to report such work in the mink (*Mustela vison*). The same year RAPACZ and SHACKELFORD published the first of a series of three preliminary reports on immunogenetic investigations begun in 1961 (RAPACZ and SHACKELFORD 1962, 1963a,b). The inheritance of seven blood group factors determined by genes at four loci is reported here.

TECHNIQUES

Reagents for the seven factors function as agglutinins. They were used in saline agglutination tests for detecting blood antigens designated A, B, B₂, C, D, E and G. One drop of a 5% suspension of mink red cells in 0.9% saline was added to a tube containing two drops of the reagent. Tubes prepared in this manner were shaken, allowed to remain at room temperature for 20 to 45 minutes, centrifuged 2 minutes at 1,500 r.p.m., and the degree of agglutination recorded: complete agglutination was indicated by +++++, while ++++, ++, + and ± were used for descending degrees of reaction. A gamma-globulin test was used to detect the D factor at the outset for reasons to be explained shortly. Additional details of the techniques and procedures used in this study have been reported elsewhere (RAPACZ and SHACKELFORD 1963a, b).

RESULTS

A system: Reagents for detecting blood factors A, B, B₂ and C were derived from isoimmune, heteroimmune and normal mink sera. (Only mink vaccinated against virus enteritis have been found with natural antibodies [RAPACZ and SHACKELFORD 1966b].) Anti-A and -B preparations were obtained from chicken and rabbit anti-mink red cells sera, but an anti-A reagent could not be obtained from ferrets. Later it was found that the 40 ferrets tested were characterized by an antigen similar to the A of mink. Anti-B₂ reagents were prepared from normal and isoimmune and ferret anti-mink sera. Anti-C was derived from normal and immune mink sera only.

All individuals except two littermate sisters of the 2200 animals tested from the University herd and two large commercial ranches in Wisconsin (R. G. STEPHENSON RANCH, Cedarburg, and WERTH's Capitol Fur Farm, Madison) fell

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TABLE 1*

Inheritance of blood factors in the A system of the mink

Type matings			Offspring				
No.	Phenotype	Genotype	No.	Exp.	Obs.	Phenotype	Genotype
33	A × A	$A^aA^a \times A^aA^a$	140	140	140	A	A^aA^a
37	A × AB	$A^aA^a \times A^aA^b$	157	78.5 78.5	74 83	A AB	A^aA^a A^aA^b
9	A × AC	$A^aA^a \times A^aA^c$	27	13.5 13.5	13 14	A AC	A^aA^a A^aA^c
25	A × B	$A^aA^a \times A^bA^b$	108	108	108	AB	A^aA^b
7	A × BC	$A^aA^a \times A^bA^c$	40	20 20	21 19	AB AC	A^aA^b A^aA^c
3	A × C	$A^aA^a \times A^cA^c$	13	13	13	AC	A^aA^c
31	AB × AB	$A^aA^b \times A^aA^b$	127	31.75 63.50 31.75	34 65 28	A AB B	A^aA^a A^aA^b A^bA^b
19	AB × AC	$A^aA^b \times A^aA^c$	67	16.75 16.75 16.75 16.75	17 17 18 15	A AB AC C	A^aA^a A^aA^b A^aA^c A^cA^c
17	AB × B	$A^aA^b \times A^bA^b$	73	36.5 36.5	36 37	AB B	A^aA^b A^bA^b
10	AB × C	$A^aA^b \times A^cA^c$	43	21.5 21.5	25 18	AC BC	A^aA^c A^bA^c
4	AC × B	$A^aA^c \times A^bA^b$	19	9.5 9.5	8 11	AB AC	A^aA^b A^aA^c
19	AC × BC	$A^aA^c \times A^bA^c$	88	22 22 22 22	21 20 29 18	AB AC BC C	A^aA^b A^aA^c A^bA^c A^cA^c
10	B × B	$A^bA^b \times A^bA^b$	31	31	31	B	A^bA^b
8	BC × BC	$A^bA^c \times A^bA^c$	42	10.5 21.0 10.5	10 21 11	B BC C	A^bA^b A^bA^c A^cA^c
2	B × BC	$A^bA^b \times A^bA^c$	10	5 5	3 7	BC B	A^bA^c A^bA^b
4	BC × C	$A^bA^c \times A^cA^c$	13	6.5 6.5	8 5	BC C	A^bA^c A^cA^c
1	B × C	$A^bA^b \times A^cA^c$	7	7	7	BC	A^bA^c
6	C × C	$A^cA^c \times A^cA^c$	24	24	24	C	A^cA^c

* Chi-square test applied where appropriate: $P \geq .2$.

into six phenogroups: A, AB(B_2), AC(B_2), B(B_2), BC(B_2) and C(B_2). Based on the serological pattern and pedigree analysis it was determined that the four red cell antigens result from genes of a triple allelic series which we have symbolized by A^a , A^b , and A^c (Table 1). The B_2 antigen shows a linear relationship with the C and B specificities while B and C bear a nonlinear relation to each other: this is interpreted to mean that B_2 is an "overlapping" specificity produced by both genes A^b and A^c . The exceptional sister pair gave a positive test to all four antigens—this appears to be the first reported case of erythrocytic antigen mosaicism in litter-bearing animals (RAPACZ and SHACKELFORD 1966a).

Blood factor D: Female 153 (A^cA^c) was the only source of reagent anti-D. Three mink and six rabbits were immunized in an effort to obtain this reagent from other sources, but without success. After the first immunization in June 1962 with red cells of mink 357 (A^cA^c) no new antibodies were detected in the serum of 153. The next December she was reimmunized and her serum showed incomplete antibodies when used in antigammaglobulin tests employing rabbit anti-mink gammaglobulin serum (RAPACZ and SHACKELFORD 1963a). Complete agglutinins which ranged in reaction from + to ++++ in a dilution of 1:4 appeared after a second reimmunization. Following this second reimmunization, 153 was bled at two week intervals for more than six months, and from 2 to 4 ml of serum obtained at each collection: during this period the titer of antibodies was maintained at the 1:4 level except for a slight rise during the latter part of pregnancy. Numerous attempts to fractionate this serum by adsorption with red cells of the various degrees of reactivity resulted in the removal of all antibodies; from this it was concluded that a single population was present and this preparation has been designated the anti-D reagent. Since some mink known by pedigree to be heterozygous give a complete reaction, degree of agglutination could not be used to determine the zygosity of an individual.

The group of 810 mink tested with the anti-D reagent came from three herds: 535 (70%) gave a positive, and 279 a negative reaction. Breeding data from all possible types of mating in this system are presented in Table 2. Twenty pairs in which both parents were negative with anti-D gave 81 offspring, all negative. In 28 matings of one parent positive and the other negative, the 132 kits were distributed in both categories. A ratio of 29 positive to 28 negative occurred in the group where the positive parent was known by pedigree to be heterozygous. Twenty-

TABLE 2
Inheritance of blood factor D in the mink

No.	Type mating				Offspring	
	Reaction to Anti-D				Reaction to Anti-D	
					+	—
20	—	×	—		0	81
12	+ (D^eD^-) *	×	—		29	28
16	+	×	—		61	14
24	+	×	+		92	10

* Known by pedigree.

four matings of positive $\times$ positive resulted in 92 positive and 10 negative young. Since mink negative for D mated together give only negative offspring, and those positive for D may produce offspring of both types of response, it is concluded that the presence of one gene, named D^d , is responsible for this blood type.

Blood factor E: The serum of rabbit R-17, immunized against type A red cells, did not contain the expected anti-A antibodies. However, after absorption with pooled red cells, it did consistently agglutinate the cells of the donor (379) of the 60 tested. No additional tests with this serum were made for several months. Later, when the sera of 320 mink were examined for naturally occurring antibodies, the serum of a single mink (T 551) gave a reaction with the red cells of one individual (379) which had been among the 60 originally tested. Further tests revealed an identical pattern of reaction in several other cases with these two sera. It was assumed that these sera, normal from mink T-551 and hetero-immune from rabbit R-17, contained the same population of antibodies: failure to further fractionate these two sera by a series of absorptions have led to the conclusion that they have a single population, and these preparations have been named reagent anti-E.

The heteroimmune serum required five absorptions, and was useful only in a dilution of 1:1. Consequently reagent anti-E from the serum of mink T-551 has been the tool of choice in continued studies since it required no absorption and could be used in dilutions of 1:8. Recently the titer has decreased to 1:4.

A group of 484 mink from three ranches were tested with the anti-E reagent, and 128 (26%) gave a positive reaction. The frequency of blood type E varied considerably between color phase groups, and in one was entirely absent.

It can be seen from the data shown in Table 3 that matings of mink negative for E produced only negative offspring: matings of positive and negative pairs produced both types of young, a near 1:1 ratio in cases where the positive parent was derived from a positive $\times$ negative mating; matings of positives gave kits the majority of which were also positive. E^e is proposed as the symbol for the gene determining positive reaction to reagent anti-E.

Blood factor G: An unexpected additional reaction was observed with red cells of mink 1329 (A^aA^a) during a test check of 60 individuals to compare a new anti- B_2 preparation from mink 334 with the original reagent. Appropriate absorptions (with B or C red cells) left antibodies against individual 1329. With this preparation, named the anti-G reagent, 361 mink were tested; 32 (8%) gave a positive, and 329 a negative reaction.

Two types of matings were available for a study of the genetics of blood type G as indicated in Table 4. The 132 offspring from parents negative for G were all negative; the positive parent in positive $\times$ negative matings was known by pedigree to be heterozygous, and approximately equal numbers of young fell into the two possible categories. Based on these observations, G^g is suggested as an appropriate symbol for the gene conditioning the red cells of a mink for a positive response to reagent anti-G.

Segregation: Only a few matings were of the appropriate genotypes for complete analysis of segregation at the four loci involved since certain genes were of

TABLE 3

Inheritance of blood factor E in the mink

No.	Type mating				Offspring	
	Reaction to Anti-E				Reaction to Anti-E +	—
36	—	×	—		0	139
15	+ (E^eE^-)*	×	—		26	32
5	+	×	—		24	4
5	+	×	+		30	1

* Known by pedigree.

low frequency or completely absent in much of our material, as has already been indicated. It was particularly difficult to make comparisons between G^g and E^e , also G^g and D^d , but less so with D^d and E^e . By contrast, it was easy to observe the relation between these three loci and genes at the A locus. Some 2,200 mink have been classified according to their blood type in the A system, 810 of these for reaction in the D system, 484 for the two preceding systems and the E system, and 359 for all four systems. Genes at the A and D loci segregate independently. For E and G the numbers were smaller, but it is clear that E^e segregates independently of the A locus and D^d . The linkage relationship of G^g with the other three loci cannot be determined with the small numbers available, although it has been observed in combination with each of the other genes.

DISCUSSION

Data on the immunogenetics of the domestic mink have been collected during the course of a two year study (RAPACZ and SHACKELFORD 1962, 1963a, b, 1966a, b). Four loci are involved in producing the seven specificities discussed. As each new factor was identified, animals already categorized with the preceding reagents were tested for their reaction to all others in so far as was possible.

Mink of the research herd were known to be highly heterozygous for genes affecting the antigenic properties of their red cells; consequently, an opportunity to observe the segregation of genes at these four loci was available. On the two commercial ranches, however, color phase groups were much more homogenous within a blood type system (especially the A system), as might be expected where each group is essentially a breeding isolate.

TABLE 4

Inheritance of blood factor G in the mink

No.	Type mating				Offspring	
	Reaction to Anti-G				Reaction to Anti-G +	—
33	—	×	—		0	132
5	+ (G^gG^-)*	×	—		12	15

* Known by pedigree.

It has been possible to test some immunogenetic relationships between the mink, the ferret and three additional Mustelids. Reagents for antigens in blood system A of the mink were used to test a limited number of the Pine (*Martes americana*), Baum (*Martes martes*) and Stone marten (*Martes foina*). All martens (35 individuals) gave a positive reaction to the anti-A reagent. Baum (4) and Stone marten (2) reacted positively with anti-C. All Pine marten (29) reacted with anti-B₂ but not with B. By contrast, the two Stone martens reacted with anti-B, but not with anti-B₂—which was never the case in mink. Two of the Baum marten reacted with anti-B₂, but none of them with anti-B. Normal mink sera gave no specific reaction with marten red cells.

SUMMARY

Seven blood factors were detected in the domestic mink by the use of agglutination tests, and designated A, B, B₂, C, D, E and G. A, B and C result from genes of a triple allelic series at the *A*^a locus; B₂ is an "overlapping" specificity produced by genes *A*^b and *A*^c: all mink tested were either homozygous for *A*^a, *A*^b, or *A*^c, or heterozygous for pairs of these three genes in all possible combinations. A single specificity was found for the *D*^d, *E*^e and *G*^g loci. Genes for the four systems are at different loci, and probably on different chromosomes.

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