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Environmental, Age, and Sex Effects on Cotton Rat (*Sigmodon Hispidus*) Hematology

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ABSTRACT: We determined the effects of sex, age, and environment (inbred, captive-wild, and wild animals) on selected blood parameters of the cotton rat (*Sigmodon hispidus*) in central Oklahoma (USA) from 1990 to 1994. Male and female cotton rats had similar blood profiles. Age-related differences were confined to differential white blood cell counts where adults possessed greater numbers of neutrophils and lower numbers of lymphocytes compared to juveniles. Environment had a strong influence on many hematological parameters. Hematocrit, hemoglobin concentration, RBC count, and eosinophil number were generally greater for wild stocks compared to inbred animals, and differences were more pronounced for adults than juveniles.

Key words: Cotton rat, *Sigmodon hispidus*, hematology, environmental effects.

The cotton rat (*Sigmodon hispidus*) is a major component of the mammalian herbivore community in grass- and brush-dominated ecosystems of the southeastern USA. The ubiquitous distribution and large size (about 150 g) of this small-mammal species has attracted the interest of ecotoxicologists who are exploring the use of biomonitors to detect ecological damage caused by human-made contaminants in the environment (Elangbam et al., 1991; Shaw-Allen and McBee, 1993; McMurry et al., 1994). Hematological analyses are routinely used in such biomonitoring efforts as an index of the general condition of the animal as well as to detect contaminant-induced pathologies. The lack of baseline information on how extrinsic and intrinsic environmental factors influence standard hematological parameters of the cotton rat has made it difficult to separate contaminant-induced changes from normal responses to their environment (McMurry, 1993).

Our objective was to determine if there were hematological differences due to age, sex, and captivity in the cotton rat. We were particularly interested in comparing hematological values among an inbred strain of cotton rats, wild animals held in captivity, and those sampled directly from the wild. Although some hematological information exists for this species, there is considerable discrepancy among studies regarding how sex, age, and captivity influence values (Hankins, 1951; Sealander, 1964; Dotson et al., 1987; Katahira and Ohwada, 1993). Differences in sample sizes and cotton rat strains (inbred vs. outbred) have been partly responsible for these discrepancies in the literature.

Wild cotton rats (n = 280) were collected 11 km west of Stillwater, Oklahoma (USA) (36°4′N, 97°11′W) from 1990 to 1994 using Sherman live-traps (H. B. Sherman Co., Tallahassee, Florida, USA) baited with peanut butter. Animals were transported back to the laboratory animal facility at Oklahoma State University, Stillwater, Oklahoma, where they were allowed to acclimate for 12 days prior to sampling blood for hematological analysis. Captive-wild cotton rats (n = 38) were animals that either were collected from the wild as described above and maintained in the laboratory animal facility for >2 mo or were the first generation of laboratory-born offspring of wild-caught rats. Inbred cotton rats (n = 91) were from our laboratory colony at Oklahoma State University, which was derived from seven breeding pairs (>30 generations removed from the wild) obtained from the National Institutes of Health. All animals were paired, maintained in polycarbonate cages with wire-tops and wood shavings for bedding,

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and fed ad libitum a diet of commercial rodent chow (Purina Mills, St. Louis, Missouri, USA) under a 14-hr light and 10-hr dark photoperiod at 23 C. Age classes were defined according to body weight: juveniles <60 g and adults ≥60 g.

Cotton rats were anesthetized with an intramuscular injection of ketamine hydrochloride (Bristol Laboratories, Syracuse, New York, USA) at a dosage of 50 mg/kg body weight prior to collecting blood from the retro-orbital sinus in 3-ml vacuum tubes containing ethylenediaminetetracetic acid (EDTA-K3) to prevent clotting. Hematocrit, red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet counts were measured on a Serono-Baker System 9000 hematology analyzer (Serono-Baker Diagnostics, Allentown, Pennsylvania, USA) using reagents and guidelines specified by the manufacturer. The instrument was previously calibrated specifically for the cotton rat using standard manual cell counting techniques. A blood smear was prepared for differential leukocyte counts, which were performed by classifying 100 cells after Wright-Giemsa staining.

Differences in hematological parameters due to sex, age, and environment (inbred, captive-wild, and wild cotton rats) were compared statistically in a three-factor unbalanced design using the General Linear Model Procedure (PROC GLM; SAS Institute Inc., 1988). Based on a Levene's test, there was heterogeneity of variance for all hematological parameters, so we rank-transformed all data prior to GLM analysis. Differences due to sex were not significant ($P > 0.05$), so data for males and females were pooled and a two-factor model (age, environment) was subsequently used for all analyses. If main factor effects were significant ($P \leq 0.05$), a Gabriel test was used to separate differences among means. Differences in monocyte and basophil numbers were not tested due to their low frequency of occurrence in blood smears.

We examined how age, sex, and environment influenced baseline values for 16 different hematological tests of cotton rats (Table 1). All individuals sampled were judged to be clinically normal. Differences between juveniles and adults were limited to differential white blood cell counts. Neutrophils comprised a greater number and lymphocytes a smaller number of the total white blood cell population in adults compared to juveniles. However, statistically significant interactions were observed as a result of more dramatic age differences among inbred animals compared to minimal differences between ages for cotton rats from wild populations. White blood cell counts and eosinophil numbers had no relationship to age class. Mean ± SE platelet numbers were slightly greater ($P < 0.03$) in adults (630 ± 13 cells × $10^3/\mu l$) compared to juveniles (585 ± 35 cells × $10^3/\mu l$).

The type of environment where the animal was raised had a profound influence on many hematological parameters (Table 1). Hematocrit, RBC count, and hemoglobin concentration values were significantly greater in cotton rats from wild populations than those from our inbred or captive-wild colonies. Differences between captive-wild and inbred stocks for these erythrocyte parameters were significant only for the RBC count, which was greatest in inbred animals. Erythrocyte indices also differed significantly among environmental types with inbred animals possessing smaller cells (MCV) that contained smaller absolute amounts (MHC) but greater concentrations (MCHC) of hemoglobin than captive-wild or wild animals. Statistical interactions were significant for all erythrocyte parameters except MCV due to greater differences among environmental types for adults than juveniles.

Total WBC counts of inbred and wild cotton rats were similar and significantly greater than counts for captive-wild ani-

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TABLE 1. Descriptive statistics for selected hematological attributes of adult and juvenile cotton rats (*Sigmodon hispidus*) sampled from inbred laboratory-raised stock, wild stock maintained in captivity, and wild populations, Oklahoma, 1990 to 1994.

	Inbred stock						Captive-wild stock						Wild stock						Statistical comparisons[a]		
	Juvenile			Adult			Juvenile			Adult			Juvenile			Adult					
Parameter	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	Age	Type	Interaction
Erythrocyte count ($\times 10^6/\mu l$)	6.2	0.2	12	5.6	0.1	76	—	—	0	4.8	0.1	23	5.7	0.2	14	6.0	0.1	226	ns	**w>i>c	**
Hemoglobin (g/dl)	11.9	0.5	12	11.0	0.1	68	—	—	0	10.5	0.2	23	12.7	0.3	12	13.4	0.1	151	ns	***w>i and c	***
Hematocrit (%)	37.1	1.5	12	34.6	0.4	80	38.2	1.1	12	34.4	0.8	26	39.0	0.8	15	41.9	0.2	263	ns	***w>i and c	***
Mean corpuscular volume (fl)	59.4	0.6	12	62.0	0.8	68	—	—	0	70.3	0.8	23	67.6	0.7	13	71.3	0.8	222	ns	***w and c>i	ns
Mean corpuscular hemoglobin (pg)	19.0	0.2	12	19.5	0.1	68	—	—	0	22.0	0.2	23	22.1	0.3	12	21.1	0.1	151	ns	***c>w>i	**
Mean corpuscular hemoglobin concentration (g/dl)	32.0	0.2	12	32.3	0.1	68	—	—	0	31.3	0.2	23	32.5	0.3	12	31.9	0.1	151	ns	**i>w>c	*
Total white blood cell count ($\times 10^3/\mu l$)	10.8	1.3	12	11.9	0.8	79	5.7	1.1	12	6.7	0.9	26	8.9	1.3	17	10.2	0.3	262	ns	***i and w>c	ns
Neutrophils ($\times 10^3/\mu l$)	3.2	1.0	12	9.2	1.0	39	2.2	0.5	6	2.9	0.8	24	3.0	0.8	11	2.8	0.2	90	**a>j	**i>c and w	**
Lymphocytes ($\times 10^3/\mu l$)	7.4	0.7	12	4.5	0.4	39	4.5	1.3	6	3.2	0.3	24	5.1	1.1	11	5.9	0.4	89	*j>a	**i and w>c	**
Monocytes ($\times 10^3/\mu l$)	0.1	0.1	12	0.0	0.0	39	0.1	0.1	6	0.1	0.1	24	0.3	0.1	11	0.1	0.1	90	—	—	—
Eosinophils ($\times 10^3/\mu l$)	0.1	0.1	12	0.3	0.1	39	0.8	0.4	6	0.4	0.1	24	0.7	0.2	11	0.5	0.1	90	ns	***w and c>i	ns
Basophils ($\times 10^3/\mu l$)	0	0	12	0	0	39	—	—	0	0	0	24	0	0	11	0.1	0.1	90	—	—	—
Platelets ($\times 10^3/\mu l$)	684	40	12	781	19	66	—	—	0	803	34	23	485	41	12	537	14	151	*a>j	***i and c>w	ns

[a] Significance (two-way analysis of variance) is indicated by * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), or ns (not significant, $P > 0.05$). Significant differences between means (Gabriel's test) are depicted by letters: a (adult), j (juvenile), c (captive-wild), i (inbred), and w (wild); —, parameter was not measured or tested.

mals; the interaction was not significant (Table 1). Based on differential white blood cell counts, the most remarkable differences among environmental types were for neutrophils, lymphocytes, and eosinophils. Neutrophils comprised a greater number of the white blood cell population of inbred cotton rats compared to captive-wild and wild-caught animals. These differences were more apparent for adults than juveniles as indicated by a significant interaction term. Lymphocyte numbers in inbred and wild cotton rats exceeded mean values of captive-wild animals; however, a significant interaction was evident because juveniles and adults appeared to respond differently to environment. Eosinophil numbers were consistently greater in blood of wild and captive-wild stocks of cotton rats than among inbred animals. Higher levels of parasitism in these wild-stock animals probably accounted for the observed differences in eosinophil numbers (Dessein and David, 1982). Basophils were rare and only observed in blood smears of wild-caught cotton rats (Table 1).

Mean values reported herein for adult cotton rats are within the range of values reported by Dotson et al. (1987) and Katahira and Ohwada (1993, males only) for laboratory-raised cotton rats, and Hankins (1951) for wild-caught animals. The genetic and environmental background of laboratory-raised cotton rats is frequently not specified in published studies. Many laboratories, including our own, routinely use laboratory-raised cotton rats from highly inbred stock, which we originally obtained from the National Institutes of Health breeding colony. Based on our results, normal baseline hematological values of cotton rats are dependent upon environmental and genetic backgrounds of animal stocks. We are aware of only one report in which such a comparison was attempted; Hankins (1951) reported that blood values of first-generation laboratory-reared animals (corresponding to our captive-wild group) did not differ from those of wild-caught cotton rats. Our results do not support this finding, perhaps partly due to sample size and genetic differences.

Several of the trends in hematological parameters of cotton rats that we observed, such as those related to oxygen supply (RBC, HGB, HCT, MCHC), are probably reflective of a more energetically active existence among wild animals compared to those raised in the laboratory, for extended periods of time. Other equally important factors accounting for some of these observed differences may be diet and season, which influence blood parameters of a variety of small mammals, including cotton rats (Sealander, 1964; McMurry et al., 1994). Animals raised in the laboratory are most often maintained on a high quality diet under constant illumination and temperature regimes. Differences in white blood cell population parameters, including platelet numbers, between wild and laboratory-raised (inbred and wild-captive) cotton rats were not surprising, given the remarkable differences in the nutritional and pathogen characteristics of their environments (Moake, 1992). However, genetic background appeared to also have an important effect on WBC subpopulations given the similarity of WBC counts between inbred and wild cotton rats in this study.

Hematological characteristics of males and females were similar for the three types of cotton rats examined in this study. However, sex influenced RBC counts, hematocrits, and MCV values in at least one inbred colony of cotton rats (Katahira and Ohwada, 1993). Sex was not observed by other investigators to influence hematological parameters of cotton rats (Hankins, 1951; Dotson et al., 1987). We propose discrepancies such as these are due at least in part to differences among studies in the degree of genetic variability and background of experimental subjects.

Age-related changes in hematological parameters of cotton rats have not been adequately documented. Hankins (1951) observed changes in median erythrocyte

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values of cotton rats varying in age from 2 to 16 mo, but did not statistically analyze these differences. Similarly, Sealander (1964) observed changes in selected erythrocyte parameters of wild cotton rats when comparing nurslings to adults but provided no statistical analysis of these differences. Although we observed no age-related differences in erythrocyte parameters, there were several effects among inbred animals, based on differential white blood cell counts. Juveniles sampled in our study ranged from 26 to 59 g body weight, so it is conceivable that age-related differences in erythrocyte parameters may be evident if younger animals are examined. The lack of remarkable age-related differences in neutrophil and lymphocyte numbers among wild stocks may be the result of stress, antigenic stimulation, inflammation, or other environmental factors.

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