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Preparing for winter: Divergence in the summer–autumn hematological profiles from representative species of the squirrel family

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Abstract

We examined hematological parameters in four related sciurid species in the late summer–autumn to assess the role of habitat, elevation, body size, and behavior in shaping these parameters. Red squirrels (*Tamiasciurus hudsonicus*) and Arctic ground squirrels (*Spermophilus parryii*) were sampled in southwestern Yukon, yellow-pine chipmunks (*Tamias amoenus*) in southern Alberta, and the eastern grey squirrel (*Sciurus carolinensis*) in southern Ontario. We obtained whole blood samples from each species and compared glucose levels, red blood cell characteristics (hematocrit, red blood cell count, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration), and white blood cell counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils) across species. We found species differences in glucose and red blood cell characteristics that may be a function of activity levels, phylogeny, or elevation, but not of body size, body condition, or adaptations to a semi-fossorial habitat. We also found species differences in white blood cell counts that remain unexplained by any single simple explanation and may be more useful for comparison of individuals within a given species than for interspecies comparisons.

Keywords: Blood; Chipmunks; Comparative hematology; Ground squirrels; Hematological standards; Rodents; Sciurids; Sciuridae; Sex differences; Tree squirrels

1. Introduction

Habitat provides the context for the evolution of life history traits that constrains and shapes successful physiological strategies (Southwood, 1977). It is not, however, the sole determinant for any particular suite of traits, with factors such as phylogenetic inertia, genetic correlation, developmental constraints, and other constraints and chance (Feder, 1987) also playing central roles. The blood is one of the major conduits for transporting a host of critical components throughout the mammalian body and thus should reflect the adaptations that permit existence in different habitats. In this paper, we examine the hematological components of four members of the squirrel family that live in a range of habitats with a diversity of life history traits.

Our objectives in this paper are threefold. First, our study is a comparative one on the hematological profiles of four representative species of the three tribes of the Sciurinae to assess how divergent life histories may have altered these profiles. These species are related members of a subfamily of the Rodentia, the most abundant of the class Mammalia (43.5% of all mammal species are rodents; Wilson and Reeder, 1993). Second, we know relatively little of the range of variation in hematological profiles within natural populations of mammals, and thus, this information is of use in understanding the basic physiological patterns in different mammalian families. Thus far, the focus of much of the hematological research on wild mammals has been on the effects of season (e.g., Scelza and Knoll, 1982; Armitage, 1983), of elevation (e.g., McLaughlin and Meints, 1972),

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and of hibernation (e.g., Nansel and Knociie, 1972; Wenberg et al., 1973), or been heavily focused on one major group, the ungulates (e.g., Nieminen, 1980; Cross et al., 1988; Delgiudice et al., 1990). Our study extends this body of knowledge by documenting the pattern of this basic physiological attribute at a specific time in the annual cycle—the crucial late summer–autumn period. Third, we describe hematological reference values for apparently healthy individuals to serve as benchmarks for future studies related to nutrition, disease, and the effects of environmental change (e.g., for similar studies, see papers on moose–Franzmann and LeResche, 1978; grizzly bears–Gau and Case, 1999; black bears–Hellgren et al., 1993; wolves–Seal et al., 1975; harbor seals–Trumble and Castellini, 2002; guanacos – Gustafson et al., 1998; dusky-footed wood rats–Weber et al., 2002).

The squirrel family (Sciuridae, order Rodentia) originated in the northern hemisphere and is well represented in North America, with over 60 species in 8 genera occupying the continent (Hafner, 1984). There are five clades in this family and these diverged in the late Eocene–early Oligocene approximately 30 million years ago and so have a long history of independent evolutionary adaptation (Mercer and Roth, 2003). We examined hematological data from two of them—the tree squirrel clade and the ground squirrel clade. Although both the behaviour and aspects of their physiology have been examined by others, baseline hematological values for many sciurids have not been published. We examined the hematology of two tree squirrel species, the red squirrel (*Tamiasciurus hudsonicus*) and the eastern grey squirrel (*Sciurus carolinensis*); a semi-fossorial squirrel, the Arctic ground squirrel (*Spermophilus parryii*); and a chipmunk, the yellow-pine chipmunk (*Tamias amoenus*). Though their common phylogenetic origin could constrain their hematological profiles (for a review of the problems and strengths of phylogenetic comparative methods, see Martins and Hansen, 1996), we expect that these members of the squirrel family are more likely to be influenced by recent selective history that is a reflection of their current and past environments. Thus, hematological profile analysis may help in the assessment of the degree of divergence of this crucial aspect of their physiology and how it plays a role in equipping these mammals for their present environment.

1.1. Life histories of sciurids

Red squirrels are tree squirrels whose distributional range covers the entire boreal forest of North America and the western Cordilleran region (Banfield, 1974; Steele, 1998). They are small (ca. 250 g), diurnal granivores specializing in seeds from spruce and pine (Obbard, 1987), but also eating a variety of other vegetable and animal matter. They defend individual, non-overlapping territories year-round (Price et al., 1990), and reproductive activity occurs from late February to July.

Eastern grey squirrels are larger (ca. 500 g) than red squirrels, but are also diurnal granivores (Thompson, 1977a; Koprowski, 1994). They are found primarily in deciduous forests throughout the eastern United States and southeastern Canada, but are also common in urban settings throughout their natural range and have now been released in a number of cities in western Canada. They are not territorial, and home ranges overlap extensively with conspecifics as well as with those of red squirrels where the ranges of the two species overlap. Reproductive activity in southern Ontario peaks in late January and early June (Thompson, 1977b). They are active throughout the winter, eating food items stored in hundreds of locations throughout their home range.

Yellow-pine chipmunks are small (ca. 50 g), semi-fossorial, diurnal granivores found in mountainous regions of the southwestern Canada and northwestern United States, primarily in open forests (Sutton, 1992). They are not territorial, but individuals maintain a home burrow that they defend from conspecifics. They remain in their burrows over winter, hibernating for 10-day periods between which they rouse briefly to eat from a single cache of food stored in the burrow. Reproduction takes place in spring, and from July onwards, they are focused on provisioning their underground food cache.

Arctic ground squirrels are found primarily in Arctic Alpine and mainland Arctic tundra of North America, but in the northwest are also found in grassland areas within the boreal forest (Banfield, 1974), where their range overlaps that of the red squirrel. Arctic ground squirrels are medium-sized (ca. 500–750 g), diurnal burrowing herbivores that eat a large variety of above-ground vegetable matter, but also animal matter when available (McLean, 1985; Boonstra et al., 1990; O'Donoghue, 1994). Reproduction takes place in spring, and by mid-summer all animals are focused on securing winter hibernacula and on building fat reserves, though males also cache seeds.

Thus, we are comparing two arboreal, non-hibernating species and two semi-fossorial, hibernating species. If habitat plays a major role in shaping hematological profiles, there may be a divergence based on these differences. Red and grey squirrels are more closely related to each other than to either of the other two species (Herron et al., 2004), they share similar ecological niches, and both remain active throughout the winter. Thus, we expected their hematology to be most similar. We also expected that the two hibernating species to be similar as they are both adapted to winter hibernation in burrows, thus, should share similar adaptations in hemoglobin structure (Clementi et al., 2003) and gene expression (Takamatsu et al., 1993; Takamatsu et al., 1997; Ono et al., 2003) that the non-hibernating tree squirrels do not. Though ground squirrels store food internally as body fat, whereas chipmunks store food in their burrow, neither species at the time of collection was metabolizing body fat for energy so their different storage strategies should not affect their hematological profiles. Seasonal changes in physiology to prepare for various annual functions (reproduction, preparation for winter, etc.) are the norm (e.g., Perez-Suarez et al., 1990; Rosenmann and Ruiz, 1993; Kenagy and Place, 2000; Place and Kenagy, 2000; Lincoln et al., 2003), and these changes should be reflected in the hematology. To standardize among species, we only examined animals in late summer, after reproduction was over and preparation for winter was underway. We did not expect sex differences in the chipmunks or red or grey squirrels, since all animals were collected outside of their normal breeding season and both sexes were in similar situations. Male and female chipmunks and grey squirrels were both collecting and storing food for the winter, and were non-territorial. All red squirrels were also engaged in food collection and storage, though animals of both sexes in this species remained highly territorial. In Arctic ground squirrels, however, the males and females were dealing with subtly different circumstances, since the males had to continue defending their territories while collecting and storing food for early spring consumption, whereas the females are non-territorial and rely solely on fat stores for winter survival and emerge from hibernation only after food becomes available in the spring.

2. Materials and methods

2.1. Trapping and sample collection

Red squirrels were trapped in Tomahawk live-traps (14 × 14 × 40 cm or 16.5 × 16.5 × 48 cm; Tomahawk Live Trap Company, Tomahawk, WI, USA) in August 1998, 1999, and 2003 in the Kluane Lake region of Yukon, Canada (within northern boreal forests of primarily white spruce; elevation approximately 1000 m). Arctic ground squirrels were also trapped in Tomahawk live-traps in August 1998 and 1999 in the same area. Eastern grey squirrels were trapped in Tomahawk live-traps in October 2001 in the Rouge Valley area of Toronto, Ontario, Canada (within mixed coniferous/deciduous forest; elevation approximately 75 m). Yellow-pine chipmunks were trapped in Longworth live-traps (Penlon-Britannia Medical Supplies, Mississauga, Ontario, Canada) in September 2001 in the Kananaskis Valley of the front ranges of the Rocky Mountains in southwest Alberta, Canada (at the edges of subalpine forests near creek beds; elevation approximately 1400 m). All animals were trapped and sampled by the same researcher (RB), and all animals were in traps for less than 3 h before blood samples were taken.

Upon removal from the trap, animals were anaesthetized using isoflurane, USP delivered at 3.5% in air from a purpose-built portable anaesthetic delivery unit and weighed using a Pesola spring-scale to the nearest 1 g (chipmunks) or 5 g (ground and tree squirrels). As part of a different study, many of the red squirrels were released after collection of a blood sample; the sacrificed animals (including all grey squirrels and chipmunks) were euthanized using an overdose of anaesthetic and the blood sample collected within 30 s from the left ventricle of the heart (cardiac puncture). Blood from ground and released red squirrels was sampled from the suborbital sinus using a glass pipette coated with heparin.

2.2. Blood analyses

Whole blood glucose concentrations were determined using AccuCheck glucometers (AccuCheck III, Mannheim Boehringer, Mannheim, Germany), except for red squirrels trapped in 2003 for which FreeStyle glucometers were used (TheraSense, Alameda, CA). Whole blood samples from red squirrels captured in 2003, grey squirrels and chipmunks were sent in 0.5 mL vials to Vita-tech Veterinary Laboratory Services (Markham, Ontario, Canada) for determination of red blood cell (RBC) concentration, hematocrit (packed red blood cell volume), hemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell (WBC) concentration, including concentrations of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. For ground squirrels and red squirrels captured in 1998–1999, hematocrit was determined by centrifuging blood samples in microcapillary tubes and differential leukocyte counts were taken on dried blood smears stained with Wright's stain. The ratio of neutrophils to lymphocytes (N/L) was calculated for each animal. For all animals that were sacrificed, adrenal and testes mass were also obtained upon dissection. Body mass and examination of testes (in males) or nipple colour (in females) were used to distinguish adult from juvenile animals.

2.3. Statistical analyses

Data from juvenile animals were excluded from the analyses. No pregnant females of any species were trapped, and reproductive condition was not considered in the analyses. Data were analyzed using StatView (Caldarola et al., 1998) and expressed as means ± S.E. We tested data for homogeneity of variance using Bartlett's test; where heterogeneity of variance was detected we either transformed the data or analyzed the data non-parametrically as indicated below. If no heterogeneity of variance was detected, data were analyzed for species and sex effects using two-way ANOVA. For significant main effects ($P < 0.05$), Fisher's least-significant difference post hoc test was used to determine which means were significantly different ($P < 0.05$).

3. Results

3.1. Summary of basic animal profiles

In total, 126 adult sciurids were captured and examined in this study. None showed any obvious external or internal signs of illness or infection. All the grey squirrels, ground squirrels and chipmunks and 23 of the red squirrels (13 females and 10 males) were sacrificed (Table 1). Body mass and basic autopsy data differed between species, but more informative was that the existence of sex differences, the direction of sex differences, and the comparisons of autopsy data corrected for body mass (Table 1). Males and females were of similar body mass in grey squirrels ($F_{1,12}=1.68$; $P=0.22$) and in red squirrels ($F_{1,61}=1.84$; $P=0.18$). However, male ground squirrels were 14% heavier than female ground squirrels ($F_{1,30}=6.42$; $P=0.02$), whereas female chipmunks were 9% heavier than male chipmunks ($F_{1,15}=6.43$; $P=0.02$).

Similar results were obtained for total adrenal mass: males and females did not differ in grey squirrels ($F_{1,12}=0.03$; $P=0.87$) or in red squirrels ($F_{1,21}=0.09$; $P=0.77$). However, total adrenal mass of male ground squirrels was over twice that of female ground squirrels ($F_{1,30}=71.6$; $P<0.0001$), and total adrenal mass of female chipmunks was 20% higher than that of male chipmunks ($F_{1,15}=38.8$; $P<0.0001$). When total adrenal mass was corrected for body mass, males and females continued to be similar in grey squirrels ($F_{1,12}=0.07$; $P=0.80$) and red squirrels ($F_{1,21}=0.29$; $P=0.60$), but the sexes continued to be divergent in the other two species—higher in male than in female ground squirrels (by 81%; $F_{1,30}=42.1$; $P<0.0001$), and lower in male than in female chipmunks (by 13%; $F_{1,15}=5.22$; $P=0.04$). There were significant differences among species within both females ($F_{3,45}=88.6$; $P<0.0001$) and males ($F_{3,33}=12.3$; $P<0.0001$). Within females, grey and ground squirrels did not differ, but both had lower corrected adrenal masses than red squirrels (54–62% those of red squirrels; $P<0.0001$), which in turn had lower corrected adrenal masses than the chipmunks (75% those of the chipmunk; $P<0.0001$). Within males, grey squirrels had lower corrected adrenal masses than each of the other three species (58% those of red squirrels, 51% those of the chipmunks, and 62% those of the ground squirrels; all $P<0.0001$), and ground squirrels had lower corrected adrenal masses than the chipmunks (81% those of the chipmunks).

The testes of the male grey squirrels were scrotal; testes of the remaining species were all abdominal. After correcting for body mass, there was a significant effect of species on testes mass ($F_{3,33}=21.8$; $P<0.0001$). Corrected testes mass in grey squirrels was higher than in all other species examined (82% larger than in yellow-pine chipmunks, 163% larger than in red squirrels, and 246% larger than in ground squirrels; $P<0.0001$ for each comparison), and was higher in yellow-pine chipmunks than in ground squirrels (by 90%; $P=0.005$).

3.2. Glucose concentrations

There was a significant effect of species on blood concentration of glucose ($F_{3,72}=2.94$; $P=0.04$), but no sex effect ($F_{1,72}=0.72$; $P=0.40$) and no interaction effect ($F_{3,72}=1.62$; $P=0.19$) (Table 2). Glucose concentrations were higher in red squirrels than in ground squirrels (by approximately 22%; $P=0.04$) and in chipmunks (by approximately 29%; $P=0.02$); grey squirrels did not differ from any of the other species.

3.3. Hematocrit

Hematocrit data did not meet the homogeneity of variance assumption for ANOVA. We first analyzed the data for within-species sex differences using the Mann–Whitney *U*-test. Within Arctic ground squirrels, hematocrit was approximately 7% higher in females than in males ($P=0.01$), but there was no sex difference in grey squirrels ($P=0.07$), red squirrels ($P=0.97$), or chipmunks ($P=0.33$) (Table 2). We therefore pooled the data from both sexes for the grey, red squirrels, and chipmunks, but not for the ground squirrels, and analyzed the data for differences using the multiple Mann–Whitney *U*-test with significance set at $P<0.05/9=0.0056$. Red squirrels had higher hematocrit than ground squirrels of both sexes (by 16% compared with ground squirrel males: $P=0.0007$; by 9% compared with ground squirrel females: $P=0.0009$) and grey squirrels (by 9%; $P=0.004$), but did not differ from chipmunks ($P=0.88$). Chipmunks had higher hematocrit than ground squirrel males (by 17%; $P=0.05$) but not females ($P=0.008$).

3.4. Red blood cell parameters

There was a significant effect of species on blood concentrations of RBCs ($F_{2,73}=28.7$; $P<0.0001$) (Table 2). Concentrations were higher in chipmunks than in red

Table 1
Mean values of body descriptors in live-trapped sciurids sampled in late summer–autumn

Descriptor	Red squirrel		Grey squirrel		Chipmunk		Ground squirrel	
	Males (10)	Females (13)	Males (7)	Females (7)	Males (9)	Females (8)	Males (11)	Females (21)
Body mass (g)	244±4 (30)	238±3 (33)	615±17	587±13	52±2	56±1	632±28	553±17
Paired adrenal mass (mg)	90*±8	88*±3	135±10	133±8	22±0.4	27±1	222±16	106±6
Paired adrenal mass (mg/100g body mass)	38.4*±2.6	37.0*±1.1	22.1±1.9	22.8±1.6	43.4±1.5	49.1±2.0	35.5±2.6	19.6±1.1
Testes mass (mg)	158*±25	n/a	1063±143	n/a	49±5	n/a	320±30	n/a
Testes mass (mg/100g body mass)	65.4*±9.0	n/a	173±22	n/a	94.5±9.9	n/a	49.8±3.0	n/a

Cells show means ± 1 S.E. Sample size is indicated in parentheses below the column headings, except where indicated in parentheses in individual cells. Values marked with an asterisk (*) were determined for animals captured in 2003 only. Statistical analyses are presented in the text.

Table 2
Mean values of hematological parameters in four sciurid species

Parameter	Red squirrel		Grey squirrel		Chipmunk		Ground squirrel	
	Males (22)	Females (28)	Males (7)	Females (5)	Males (9)	Females (8)	Males (11)	Females (16)
Glucose (mmol/L)	7.4±0.9 (11)	6.4±0.7 (13)	5.8±0.6 (6)	7.9±0.6 (4)	5.4±0.4	5.2±0.8	5.1±0.4	6.0±0.5 (13)
Hematocrit (%)	49.7±1.4 (30)	50.0±1.1 (32)	46.3±0.8	44.6±0.5	48.4±2.0	51.8±1.6	42.8±1.0	45.9±0.7 (21)
RBC (10^{12} cells/L)	8.44*±0.20	7.81*±0.17	6.90±0.22	6.76±0.15	9.01±0.36	9.65±0.30		
Hemoglobin (g/L)	156*±3	153*±2	137±2	135±2	145±6	160±3		
MCV (fL)	63.3*±1.2	66.3*±1.0	67.4±1.2	66.2±0.9	54.0±1.3	53.9±1.0		
MCH (pg)	18.6*±0.2	19.8*±0.2	19.9±0.3	20.2±0.4	16.4±0.3	16.6±0.4		
MCHC (g/L)	293*±4	298*±3	296±3	304±3	300±4	309±6		
WBC (10^9 cells/L)	1.74*±0.34	1.86*±0.26	5.07±0.99	6.00±1.68	2.50±0.37	2.48±0.57		
Neutrophils (%)	59.9±4.2 (28)	58.4±3.6 (32)	59.5±6.5	71.0±4.6	21.0±2.9	18.3±2.2	46.8±4.6	37.9±5.4
Neutrophils (10^9 cells/L)	1.09*±0.25	1.13*±0.19	3.30±0.96	4.43±1.41	0.47±0.06	0.44±0.12		
Lymphocytes (%)	38.9±4.2 (28)	40.7±3.7 (32)	34.6±7.6	27.6±4.6	69.8±3.4	73.6±2.7	31.6±4.9	45.1±6.0
Lymphocytes (10^9 cells/L)	0.65*±0.12	0.73*±0.12	1.46±0.38	1.53±0.34	1.82±0.36	1.83±0.43		
N/L	4.63±1.70 (28)	3.16±0.95 (32)	3.36±1.62	2.87±0.45	0.32±0.06	0.26±0.04	2.01±0.40	2.73±1.58
Monocytes (%)	1.11±0.46 (28)	0.88±0.56 (32)	2.04±0.85	0.20±0.20	2.81±0.39	2.04±0.20	18.91±3.52	15.19±2.62
Monocytes (10^9 cells/L)	0*	0*	0.10±0.05	0.01±0.01	0.06±0.005	0.05±0.01		
Eosinophils (%)	0.04±0.04 (28)	0 (32)	0.74±0.25	1.20±0.58	1.41±0.54	2.10±0.41	2.64±0.64	2.25±0.52
Eosinophils (10^9 cells/L)	0*	0*	0.03±0.01	0.04±0.02	0.03±0.01	0.05±0.01		
Basophils (%)	0 (28)	0 (32)	0	0	0.93±0.17	1.31±0.24	0	0
Basophils (10^9 cells/L)	0*	0*	0	0	0.02±0.004	0.03±0.01		

Cells show means ± 1 S.E. for each sex. Sample size is indicated in parentheses below the column headings, except where indicated in parentheses in individual cells. Values marked with an asterisk (*) were determined for animals captured in 2003 only. Statistical analyses are presented in the text. Abbreviations: RBC – red blood cell count; MCV – mean corpuscular volume in femtoliters; MCH – mean corpuscular hemoglobin in picograms; MCHC – mean corpuscular hemoglobin concentration; WBC – white blood cell count; N/L – neutrophil/lymphocyte ratio. Abbreviations: MCH (pg) – mean corpuscular hemoglobin in picograms; MCHC – mean corpuscular hemoglobin concentration; MCV (fL) – mean corpuscular volume in femtoliters; N/L – neutrophil/lymphocyte ratio; RBC – red blood cell count; WBC – white blood cell count.

squirrels (by 15%; $P<0.0001$) and in grey squirrels (by 36%; $P<0.0001$), and higher in red squirrels than in grey squirrels (by 18%; $P<0.0001$). There was no effect of sex on RBC concentration ($F_{1,73}=0.03$; $P=0.86$), but there was a significant interaction effect ($F_{2,73}=3.35$; $P=0.01$). When t-tests were run on each species separately, male and females were similar in grey squirrels ($t_{10}=0.48$; $P=0.64$) and in chipmunks ($t_{15}=1.35$; $P=0.02$), but male red squirrels had 8% higher RBC concentrations than females ($t_{48}=-2.44$; $P=0.02$).

Hemoglobin data did not meet the homogeneity of variance assumption for ANOVA. We first analyzed the data for within-species sex differences using the Mann–Whitney U-test. There was no sex difference in grey squirrels ($P=0.42$), red squirrels ($P=0.48$), or chipmunks ($P=0.07$). We therefore pooled the data from both sexes in each species and analyzed the data for species differences using the multiple Mann–Whitney U-test with a correction for significance with P set at $0.05/3=0.017$. Red squirrels had higher hemoglobin concentrations than those in grey squirrels (by 13%; $P<0.0001$), but values did not differ from those in chipmunks ($P=0.89$). There was no significant difference between grey squirrels and chipmunks ($P=0.002$).

For mean corpuscular volume (MCV), there was a significant effect of species ($F_{2,73}=36.7$; $P<0.0001$). Red blood cells were smaller in chipmunks than in grey (by 24%; $P<0.0001$) and red squirrels (by 20%; $P<0.0001$). There was no sex effect on MCV ($F_{1,73}=0.18$; $P=0.68$), and no interaction effect ($F_{2,73}=1.28$; $P=0.28$). For mean corpuscular hemoglobin (MCH), there was also a significant effect of species ($F_{2,73}=56.6$; $P<0.0001$) and of sex ($F_{1,73}=4.11$; $P=0.05$), but no interaction effect ($F_{2,73}=1.75$; $P=0.18$). MCH was higher in grey squirrels than in red squirrels (by 4%; $P=0.02$) and chipmunks (by 21%; $P<0.0001$), and higher in red squirrels than in chipmunks (by 17%; $P<0.0001$). Females also had higher MCH values than males. For mean corpuscular hemoglobin concentration (MCHC), there was no significant effect of species ($F_{2,73}=2.40$; $P=0.10$), of sex ($F_{1,73}=3.08$; $P=0.08$), or of their interaction ($F_{2,73}=0.18$; $P=0.83$).

Since elevation may be one variable explaining species differences in red blood cell characteristics, we ran simple regressions with elevation and the species mean for RBC concentration, hemoglobin concentration, hematocrit, MCV, MCH, and MCHC. Adaptations to higher elevation (i.e., to decreased oxygen availability) should result in increased efficiency of oxygen transport by RBCs and should therefore include higher numbers of smaller RBCs and higher hemoglobin concentration (both total and per RBC). Though all the regressions were in the expected direction (Table 3), none were significant, possibly due to the low number of species used (3–4).

3.5. White blood cell parameters

None of the white blood cell (WBC) data (either total or for individual WBC types) met the homogeneity of variance

Table 3
Statistical results for the regression of elevation with various red blood cell characteristics in live-trapped sciurid species

RBC characteristic	*df*	F	P	r	r^2
RBC concentration	1, 2	19.9	0.14	0.98	0.95
Hemoglobin	1, 2	5.21	0.26	0.92	0.84
Hematocrit	1, 3	1.18	0.39	0.61	0.37
MCV	1, 2	2.04	0.39	−0.81	0.67
MCH	1, 2	2.73	0.35	−0.86	0.73
MCHC	1, 2	0.16	0.76	0.37	0.14

All characteristics were measured in red and grey squirrels and yellow-pine chipmunks; hematocrit was also measured in arctic ground squirrels.

assumption for ANOVA, so we either transformed the data to allow for use of ANOVA or analyzed the data using the Mann–Whitney *U*-test, as indicated below (Table 2). Total WBC data was transformed by log(x). There was a significant effect of species on blood concentrations of WBCs ($F_{2,73}$= 18.6; $P<0.0001$), but not of sex ($F_{1,73}=0.05$; $P=0.82$) or of their interaction ($F_{2,73}=0.13$; $P=0.88$). Concentrations in grey squirrels were twice as high as in chipmunks ($P=0.002$) and three times as high as in red squirrels ($P<0.0001$). Concentrations in chipmunks were higher than in red squirrels (by 38%; $P=0.02$).

Neutrophils and lymphocytes were the predominant leucocytes in the blood of each species, together constituting 80–99% of WBCs (Table 2). Neutrophils predominated in the red and grey squirrels, and lymphocytes predominated in the chipmunks and were present in roughly equal proportions in the ground squirrels. We analyzed the neutrophil/lymphocyte ratio (N/L) data for within-species sex differences using the Mann–Whitney *U*-test. There was no sex difference in any species (grey squirrels, $P=0.17$; ground squirrels, $P=0.14$; red squirrels, $P=0.74$; chipmunks, $P=0.50$). We therefore pooled the data from both sexes in each species, and analyzed the data for differences using the multiple Mann–Whitney *U*-test with significance set at $P<0.05/6=0.0083$. Grey, red, and ground squirrels had N/L of approximately 10 times those of chipmunks ($P<0.0001$); the squirrels did not differ significantly from each other (grey vs. red: $P=0.31$; grey vs. ground: $P=0.08$; ground vs. red: $P=0.15$).

The percentage of WBCs that were neutrophils was analyzed for within-species sex differences using the Mann–Whitney *U*-test. There was no sex difference in grey squirrels ($P=0.17$), ground squirrels ($P=0.22$), red squirrel ($P=0.75$), or chipmunks ($P=0.56$). We therefore pooled the data from both sexes in each species and analyzed the data for differences using the multiple Mann–Whitney *U*-test with significance set at $P<0.05/6=0.0083$. Grey squirrels and red squirrels did not differ from each other ($P=0.55$), and both had higher neutrophil percentages than ground squirrels (grey squirrels by 55%: $P=0.001$; red squirrels by 42%: $P=0.0004$) and over three times higher than chipmunks ($P<0.0001$). Ground squirrels also had neutrophil percentages that were over twice as high as chipmunks ($P=0.0001$).

Raw blood neutrophil concentration data were transformed by log(x). There was a significant effect of species ($F_{2,73}=23.0$; $P<0.0001$) but no effect of sex ($F_{1,73}=0.08$; $P=0.78$) or of their interaction ($F_{2,73}=0.40$; $P=0.68$). Neutrophil concentrations in grey squirrels were over three times those in red squirrels ($P<0.0001$) and over eight times those in chipmunks ($P<0.0001$), and concentrations in red squirrels were over twice those in chipmunks ($P=0.005$).

The percentage of WBCs that were lymphocytes was analyzed for within-species sex differences using the Mann–Whitney *U*-test. There was no sex difference in grey squirrels ($P=0.51$), ground squirrels ($P=0.13$), red squirrel ($P=0.76$), or chipmunks ($P=0.44$). We therefore pooled the data from both sexes in each species and analyzed the data for differences using the multiple Mann–Whitney *U*-test with significance set at $P<0.05/6=0.0083$. Lymphocyte proportions in chipmunks were at approximately twice those in grey, red, and ground squirrels ($P<0.0001$); the squirrels did not differ significantly from each other (grey vs. red: $P=0.21$; grey vs. ground: $P=0.45$; ground vs. red: $P=0.81$).

Raw blood lymphocyte concentration data were transformed by log(x). There was a significant effect of species on blood concentrations of lymphocytes ($F_{2,73}=21.1$; $P<0.0001$), but no significant effect of sex ($F_{1,73}=0.10$; $P=0.75$) or of their interaction ($F_{2,73}=0.09$; $P=0.92$). Lymphocyte concentrations were more than twice as high in grey squirrels and chipmunks than in red squirrels ($P<0.0001$).

Monocytes were a rare type of WBC in these rodents, constituting about 1–2% in all species except Arctic ground squirrels, in which they constituted 15–19% (Table 2). We analyzed the proportion of WBCs that were monocytes for within-species sex differences using the Mann–Whitney *U*-test. Within grey squirrels, males had monocyte concentrations 10 times higher than females ($P=0.03$), but there was no sex difference in ground squirrels ($P=0.39$), red squirrels ($P=0.36$), or chipmunks ($P=0.23$). We therefore pooled the data from both sexes for the ground and red squirrels and chipmunks, but not for the grey squirrels, and analyzed the data for differences using the multiple Mann–Whitney *U*-test with significance set at $P<0.05/9=0.0056$. Monocyte proportions were higher in ground squirrels than grey squirrels (by 8 times compared with grey squirrel males: $P=0.0001$; by over 80 times compared with grey squirrel females: $P=0.0004$), 17 times higher in ground squirrels than in red squirrels ($P<0.0001$), and 7 times higher in ground squirrels than in chipmunks ($P<0.0001$). Red squirrels did not differ from grey squirrel females, but had monocyte proportions less than half those in grey squirrel males ($P=0.0006$) and chipmunks ($P<0.0001$). Chipmunks did not differ from grey squirrel males ($P=0.13$) but had monocyte proportions 10 times higher than grey squirrel females ($P=0.0008$).

Blood monocyte concentration data were analyzed for within-species sex differences using the Mann–Whitney *U*-test. Red squirrels were excluded from the analysis as we found no monocytes in this species. Monocyte concentrations in grey squirrel males were 17 times higher than those in females ($P=0.02$), and those in chipmunk males were marginally higher than those in females ($P=0.07$). We therefore pooled the data from both sexes for the chipmunks but not the grey squirrels and analyzed the data for differences using the multiple Mann–Whitney *U*-test with significance set at $P<0.05/3=0.017$. Monocyte concentrations in chipmunks were 10 times higher than in grey squirrel females ($P=0.002$), but did not differ from those in grey squirrel males ($P=0.78$).

Eosinophils were rare in these rodents, constituting less than about 2% in all species (Table 2). As only one red squirrel had any eosinophils (composing 1% of the total number of WBCs in that individual), this species was excluded from the analysis. There was no significant effect of species on the proportion of WBCs that were eosinophils ($F_{2,50}=3.02$; $P=0.06$), of sex ($F_{1,50}=0.26$; $P=0.61$) or of their interaction ($F_{2,50}=0.56$; $P=0.57$). Since the species effect was nearly significant, we ran Fisher's post hoc test, as there may be a biologically significant species difference that was masked by inadequate sample size (power for the species effect was 0.55). The post hoc test suggested that percentages of eosinophils were over twice as high in ground squirrels than in grey squirrels ($P=0.02$). Blood eosinophil concentrations were analyzed for grey squirrels and chipmunks only; there was no significant effect of species ($F_{1,25}=0.04$; $P=0.84$) of sex ($F_{1,25}=0.65$; $P=0.43$), or of their interaction ($F_{1,25}=0.16$; $P=0.70$).

Basophils were the rarest WBC type in these rodents, being found only in chipmunks (about 1%, Table 2). Within chipmunks, there was no significant effect of sex on basophil percentage ($F_{1,15}=1.72$; $P=0.21$). Raw blood basophil concentration data were transformed by sqrt(x). There was no difference between the sexes ($t_{15}=0.84$; $P=0.41$).

4. Discussion

4.1. Summary of major patterns

In this study, we document the hematological values from apparently healthy individuals of four rodent species of the subfamily Sciurinae at one biologically equivalent point in time: late summer–early autumn. What is striking about the hematological data (Table 2) is that although many of the values are approximately similar (even those that are statistically different, i.e., MCV, MCHC, glucose levels), a number of the values differ markedly across species (e.g., RBCs are 15% and 36% higher in chipmunks than red squirrels and grey squirrels, respectively; WBCs are two and three times higher in grey squirrels than in chipmunks and red squirrels, respectively). Some of these differences may be a function of phylogeny (common descent), but this cannot be distinguished in our study from evolutionary adaptations to living in a similar habitat (e.g., both red and grey squirrels are arboreal; chipmunks and Arctic ground squirrels are semi-fossorial). Excluding the rarest white blood cell types (monocytes, eosinophils, and basophils), red squirrels were more similar to grey squirrels (statistical differences notwithstanding) than they were to chipmunks and Arctic ground squirrels in 9 of 11 variables (Table 2). The similarity between chipmunks and Arctic ground squirrels is less striking—these two species are more similar to each other than to red and grey squirrels in 3 of 5 variables—but fewer hematological values were available for comparison, so we cannot reject the hypothesis that habitat has helped shape the evolution of hematological traits.

4.2. Caveats

One caveat should be mentioned. All our animals were subjected to trapping prior to blood sampling, so differences may reflect species-specific responses to trapping stress (increased glucose levels, increased hematocrit, etc.), rather than species differences per se. For example, the spleen, a storage site for erythrocytes, contracts in response to an adrenalin surge caused by stress and releases erythrocytes into the blood (Guthrie et al., 1967; Cross et al., 1988; Delgiudice et al., 1990), causing a temporary increase in hematocrit. Trapping and handling is known to stress Arctic ground squirrels (Boonstra et al., 2001a) and chipmunks (Kenagy and Place, 2000; Place and Kenagy, 2000), and thus, the hematocrit levels we obtained were those of animals experiencing stress. In addition to hematological differences, the species also differed as to whether males and females differed in body and adrenal mass (Table 1).

4.3. Species differences in body mass

There were differences among the species in the various physical mass measurements collected; all our body mass data were within reported species norms (red squirrels–Steele, 1998; grey squirrels–Koprowski, 1994; chipmunks–Sutton, 1992; ground squirrels–Wilson and Ruff, 1999). Whereas there was no difference between the sexes in grey or red squirrels, male ground squirrels and female chipmunks were heavier and had larger adrenals than conspecifics of the opposite sex. In ground squirrels, the difference in adrenal mass is most likely due to continued intense competition for access to hibernacula near those of females (Carl, 1971; Lacey and Wieczorek, 2001). Such hibernacula allow easier access to females when they become reproductively active in the spring, but the competition for such burrows causes males increased stress and thus would increase adrenal mass. Likewise, in ground squirrels the larger body size of males is expected because of physical male–male competition in the spring for mates (Lacey, 1991). In yellow-pine chipmunks, males may be smaller than females because small males aggressively dominate larger males (Schulte-Hostedde and Millar, 2002), whereas larger females are reproductively more successful when sufficient resources are available to maintain their larger body size (Schulte-Hostedde et al., 2002). Whereas larger adrenals might be expected in female chipmunks due to larger body size, the difference between the sexes was not accounted for by body size alone, since females also had larger adrenal mass as expressed per 100 g of body mass.

4.4. Glucose

Glucose values were highest in red squirrels, followed by grey squirrels, ground squirrels, and chipmunks (Table 2). These differences are not simply a result of different body mass. Though red squirrels had the highest glucose levels and were smaller than both grey and Arctic ground squirrels, the chipmunks had the lowest glucose levels, yet were the smallest species. The species differences we found may reflect differences in baseline glucose levels. For grey squirrels, the glucose levels we obtained were similar to those obtained from shot animals (6.03 mM in males, and 6.29 mM in females: Guthrie et al., 1966), so trapping may either provoke a minimal stress response compared with that which is typically evoked by handling (e.g., Kenagy and Place, 2000; Place and Kenagy, 2000) or hormonal stress protocols (e.g., Boonstra and McColl, 2000) or may be attenuated with the length of time spent in the trap (up to 3 h). Alternatively, if trapping does act as a salient stressor, and since blood glucose rises in response to acute stress within minutes (Dallman et al., 1989), these values may reflect different responses to trapping stress. Chipmunks may have experienced less stress than the other species and thus experienced a less intense rise in blood glucose levels, since the traps used for this species were enclosed whereas the traps for the other species were open wire traps that allowed the animals to see—and be seen from—the environment outside the trap. Trapped Arctic ground squirrels appear much less perturbed than tree squirrels while in a trap; they tend to stand quietly in the trap whereas both red and grey squirrels frequently vocalize and forcefully throw themselves at the trap walls in an effort to escape. Based on this behaviour, we expected ground squirrels to display fewer physiological signs of stress than tree squirrels and thus show lower glucose levels. Whereas grey squirrels are non-territorial, red squirrels' survival depends on defense of their territory against invasion by nearby red squirrels, and a removed squirrel can lose its territory to rivals within a few hours (S. Boutin, unpublished observations). Since being in a trap prevents them from adequately defending their territory against invaders, red squirrels should show more physiological signs of stress, and thus higher glucose levels, than non-territorial grey squirrels in response to trapping.

4.5. Red blood cell parameters

Unlike blood glucose levels, there appeared to be no systematic differences in red blood cell parameters. There are a number of possible explanations, none of which explain all the differences found. One potential explanation is differences in the elevations at which individuals of each species were captured, since animals living at higher elevations may adapt to the lower oxygen pressure in the air by increasing the production of red blood cells and of the amount of hemoglobin, and by a change in the physiochemical properties of the hemoglobin molecule enabling it to take in and unload oxygen more readily (Hock, 1970; Marshall and Matthias, 1971; Monge and Whittembury, 1974). However, none of our regressions of RBC parameters and elevation were statistically significant, though we had low power as we only examined three to four species. A second explanation is that differences may be a reflection of nutritional condition and of exposure to chronic stressors. Higher hematocrit values indicative of better nutritional condition have been seen in a number of species (moose–Franzmann and LeResche, 1978; collared peccaries–Lochmiller et al., 1986; black bears–Hellgren et al., 1993), and lower values indicative of exposure to chronic stress (i.e., high predation risk) have been seen in snowshoe hares during the decline phase of the 10-year cycle (a difference of 6% from the decline phase to the low phase–Boonstra et al., 1998) and in breeding male Arctic ground squirrels (i.e., a 6% decline during spring mating relative to nonbreeding males–Boonstra et al., 2001b). However, since all the animals appeared healthy and in good overall condition, this explanation is unlikely; in addition, due to their proximity to humans and therefore potentially supplementary food, the grey squirrels were the most likely of all the species examined to be in better than average condition, and yet they had the second-lowest hematocrit of the four species we examined. The grey squirrels we examined did have higher hematocrit levels than in shot grey squirrels in another study (40.8% in males and 32.5% in females; Guthrie et al., 1966), but low values may have been due to postmortem clotting within the circulation, decreasing the concentration of RBCs in the remaining liquid blood. Additionally, other values we obtained from trapped grey squirrels were very similar to those obtained from shot animals (e.g., Hb 93–16.3 g/L, MCHC 288–400 g/L–Guthrie et al., 1966). Finally, a third potential explanation involves adaptations to high oxygen demands. Contrary to our initial expectation that red and grey squirrels should possess the most efficient oxygen transport systems, since they appear more physically active than ground squirrels and chipmunks and thus were expected to require higher blood oxygen levels, neither of the tree squirrel species had all the features necessary for extremely efficient oxygen transport, i.e., a relatively large number of small RBCs with high hemoglobin concentration. Alternatively, it could be expected that burrowing species (chipmunks and ground squirrels) should possess the more efficient oxygen transport systems, since they are exposed to depleted oxygen levels underground (Armitage, 1983). But again, neither of these two species, relative to the tree squirrels, had all the characteristics of extremely efficient oxygen transport. Additionally, in a third semi-fossorial sciurid species, the yellow-bellied marmot (*Marmota flaviventris*) (Armitage, 1983), though they are more similar to the chipmunks than to the tree squirrels we examined in hematocrit values (mean in yellow-bellied marmots: 50.8%), these marmots are more similar to one of the two tree squirrel species than to the chipmunks in RBC count (mean in marmots: 6.16×10^{12} cells/L), MCV (mean in marmots: 73.3 fL), MCH (mean in marmots: 23.7 pg), MCHC (mean in marmots: 264 g/L), and WBC count (mean in marmots: 9.41×10^{9} cells/L). Thus, habitat (arboreal vs. semi-fossorial) does not appear to determine hematological characteristics.

We had expected grey and red squirrels to be very similar to each other, since they are closely related phylogenetically and occupy similar ecological niches. However, they differed from each other in several variables. These differences may be due in part to male grey squirrels having scrotal testes, whereas the male red squirrels' were all abdominal, although both species were collected outside their breeding seasons. In spite of this, the only differences found between grey squirrel males and females were in monocyte concentration and proportion, where males had higher counts for both (though the difference between the sexes in hematocrit may be biologically relevant, perhaps due to low sample size it was not statistically significant). The lack of difference between male and female grey squirrels in any other variable argues against the males' scrotal testes being at the root of the differences between red and grey squirrels. Whereas species-specific data on red blood cells may be valuable for determining whether detrimental conditions exist for a given population, such as a shortage of water or food, the interspecies comparison in this case did not reveal any apparent pattern that would generalize to other species. The four different species examined in the current study have different nutritional needs and may differ in their exposure to stressors (including, for example, predators and weather), so it is not possible to draw conclusions here regarding the contribution of each of these parameters to the determination of optimal RBC characteristics.

4.6. White blood cell parameters

Species differences were found in the proportions of white blood cells that were comprised of the various cell subtypes. In proportions of the various white blood cell types, red and grey squirrels were more similar to each other than to either of the other species examined. Neutrophils, as the "first line of defense" against infection, are released in response to stress, so the relatively high proportions of neutrophils present in red and grey squirrels may be part of the stress response that also resulted in elevated blood glucose levels. In contrast to the tree squirrels, chipmunks had the lowest proportion of neutrophils and highest proportion of lymphocytes (which decrease in response to stress), again suggesting that of all four species they were minimally stressed by trapping, with ground squirrels again falling between the tree squirrels and the chipmunks. The reason for the presence of basophils only in chipmunks is unknown, but as both the proportion and absolute concentration of these cells in chipmunks is low, their presence is likely to be normal in this species, and their apparent absence in the other species may be due to a lack of detection rather than to complete absence of these cells.

In terms of concentration, red squirrels had the lowest total concentration of white blood cells and grey squirrels the highest. Red squirrels therefore had lower absolute concentrations of each white blood cell type than grey squirrels. Despite this difference, chipmunks still had the lowest concentration of neutrophils and the highest concentration of lymphocytes. We did not obtain absolute concentrations of each white blood cell type in Arctic ground squirrels, but we would expect their values to again fall between those of the chipmunks and the tree squirrels.

4.7. Conclusions

In conclusion, we report the hematological values from four related members of the tribe Sciurinae that range in habitat from being burrowing to arboreal, and in activity from hibernating up to 75% of the year to being active year round. Though there are broad-scale similarities in the patterns of some of the variables, marked divergence occurs in others. A number of adaptive explanations are possible to explain these patterns, though the conclusions could be strengthened by collecting data on a larger number of related species. For the present, we can exclude body size and condition, and adaptations specific only to oxygen demands as likely explanations. We cannot reject the hypotheses that activity, phylogeny, or elevation are explanatory mechanisms. Finally, we do not currently have any data to explain the marked differences in WBC and in the leukocyte concentrations and proportions in these sciurid species.

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References

Armitage, K.B., 1983. Hematological values for free-ranging yellow-bellied marmots. Comp. Biochem. Physiol. A 74, 89–93.

Banfield, A.W.F., 1974. The Mammals of Canada. National Museum of Canada and University of Toronto Press, Toronto.

Boonstra, R., McColl, C.J., 2000. Contrasting stress response of male Arctic ground squirrels and red squirrels. J. Exp. Zool. 286, 390–404.

Boonstra, R., Krebs, C.J., Kanter, M., 1990. Arctic ground squirrel predation on collared lemmings. Can. J. Zool. 68, 757–760.

Boonstra, R., Hik, D.S., Singleton, R.S., Tinnikov, A., 1998. The impact of predator-induced stress on the snowshoe hare cycle. Ecol. Monogr. 68, 371–394.

Boonstra, R., Hubbs, A.H., Lacey, E.A., McColl, C.J., 2001a. Seasonal changes in glucocorticoid and testosterone concentrations in free-living Arctic ground squirrels from the boreal forest of the Yukon. Can. J. Zool. 79, 49–58.

Boonstra, R., McColl, C.J., Karels, T.J., 2001b. Reproduction at all costs: the adaptive stress response of male Arctic ground squirrels. Ecology 82, 1930–1946.

Caldarola, J., Dilmaghani, A., Gagnon, J., Haybock, K., Roth, J., Soper, C., Wasserman, E., 1998. StatView 5.0.1. SAS Institute, Cary, NC.

Carl, E.A., 1971. Population control in Arctic ground squirrels. Ecology 52, 395–413.

Clementi, M.E., Petruzzelli, R., Filippucci, M.G., Capo, C., Misiti, F., Giardina, B., 2003. Molecular adaptation to hibernation: the hemoglobin of *Dryomys nitedula*. Eur. J. Physiol. 446, 46–51.

Cross, J.P., MacKintosh, C.G., Griffin, J.F.T., 1988. Effects of physical restraint and xylazine sedation on haematological values in red deer (*Cervus elaphus*). Res. Vet. Sci. 45, 281–286.

Dallman, M.F., Darlington, D.N., Suemaru, S., Cascio, C.S., Levin, N., 1989. Corticosteroids in homeostasis. Acta Physiol. Scand. 136, 27–34.

Delgiudice, G.D., Kunkel, K.E., Mech, L.D., Seal, U.S., 1990. Minimizing capture-related stress on white-tailed deer with a capture collar. J. Wildl. Manage. 54, 299–303.

Feder, M.E., 1987. The analysis of physiological diversity: the prospects for pattern documentation and general questions in ecological physiology. In: Feder, M.E., Bennett, A.F., Burggren, W.W., Huey, R.B. (Eds.), New Directions in Ecological Physiology. Cambridge University Press, Cambridge, UK, pp. 38–70.

Franzmann, A.W., LeResche, R.E., 1978. Alaskan moose blood studies with emphasis on condition evaluation. J. Wildl. Manage. 42, 334–351.

Gau, R.J., Case, R., 1999. Evaluating nutritional condition of grizzly bears via select blood parameters. J. Wildl. Manage. 63, 286–291.

Gustafson, L.L., Franklin, W.L., Sarno, R.J., Hunter, R.L., Young, K.M., Johnson, W.E., Behl, M.J., 1998. Predicting early mortality of newborn guanacos by birth mass and hematological parameters: a provisional model. J. Wildl. Manage. 62, 24–35.

Guthrie, D.R., Mosby, H.S., Osborne, J.C., 1966. Hematological values for the Eastern Gray squirrel (*Sciurus carolinensis*). Can. J. Zool. 44, 323–327.

Guthrie, D.R., Osborne, J.C., Mosby, H.S., 1967. Physiological changes associated with shock in confined gray squirrels. J. Wildl. Manage. 31, 102–108.

Hafner, D.J., 1984. Evolutionary relationships of the nearctic sciuridae. In: Murie, J., Michener, G. (Eds.), The Biology of Ground-Dwelling Squirrels. University of Nebraska Press, Lincoln, pp. 1–23.

Hellgren, E.C., Rogers, L.L., Seal, U.S., 1993. Serum chemistry and hematology of black bears: physiological indices of habitat quality or seasonal patterns? J. Mammal. 74, 304–315.

Herron, M.D., Castoe, T.A., Parkinson, C.L., 2004. Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (*Spermophilus*). Mol. Phylogenet. Evol. 31, 1015–1030.

Hock, R.J., 1970. The physiology of high altitude. Sci. Am. 22, 52–62.

Kenagy, G.J., Place, N.J., 2000. Seasonal changes in plasma glucocorticosteroids of free-living female yellow-pine chipmunks: effects of reproduction and capture and handling. Gen. Comp. Endocrinol. 117, 189–199.

Koprowski, J.L., 1994. *Sciurus carolinensis*. Mamm. Species 480, 1–9.

Lacey, E.A., 1991. Reproductive and dispersal strategies of male Arctic ground squirrels (*Spermophilus parryii plesius*). PhD thesis. University of Michigan, Ann Arbor.

Lacey, E.A., Wieczorek, J.R., 2001. Territoriality and male reproductive success in Arctic ground squirrels. Behav. Ecol. 12, 626–632.

Lincoln, G.A., Andersson, H., Loudon, A., 2003. Clock genes in calendar cells as the basis of annual timekeeping in mammals: a unifying hypothesis. J. Endocrinol. 179, 1–13.

Lochmiller, R.L., Hellgren, E.C., Varner, L.W., Grant, W.E., 1986. Serum and urine biochemical indicators of nutritional status in adult female collared peccaries, *Tayassu tajacu* (Tayassuidae). Comp. Biochem. Physiol. A 83, 477–488.

Marshall, L.G., Matthias, D.V., 1971. Correlations between physiological parameters of blood and altitude in *Peromyscus maniculatus*. J. Mammal. 52, 449–450.

Martins, E.P., Hansen, T.R., 1996. The statistical analysis of interspecific data: a review and evaluation of phylogenetic comparative methods. In: Martins, E.P. (Ed.), Phylogenies and the Comparative Methods in Animal Behavior. Oxford University Press, New York, NY, pp. 22–75.

McLaughlin, D.W., Meints, R.H., 1972. A study of hibernator erythropoietic responses to stimulated high altitude. Comp. Biochem. Physiol. A 42, 655–666.

McLean, I.G., 1985. Seasonal patterns and sexual differences in the feeding ecology of Arctic ground squirrels (*Spermophilus parryii plesius*). Can. J. Zool. 63, 1298–1301.

Mercer, J.M., Roth, V.L., 2003. The effects of cenozoic global change on squirrel phylogeny. Science 299, 1568–1572.

Monge, C., Whittembury, J., 1974. Increased hemoglobin-oxygen affinity at extremely high altitudes. Science 186, 843.

Nansel, D., Knociie, L., 1972. Blood changes in torpid and non-torpid Columbian ground squirrels, *Spermophilus columbianus*. Comp. Biochem. Physiol. A 41, 175–179.

Nieminen, M., 1980. Nutritional and seasonal effects on the haematology and blood chemistry in reindeer (*Rangifer tarandus tarandus L.*). Comp. Biochem. Physiol. A 66, 399–413.

Obbard, M.E., 1987. Red squirrel. In: Novak, M., Obbard, M.E., Malloch, M.E. (Eds.), Wild Furbearers: Management and Conservation in North America. Ontario Ministry of Natural Resources, Toronto, Canada, pp. 265–281.

O'Donoghue, M., 1994. Early survival of juvenile snowshoe hares. Ecology 75, 1582–1592.

Ono, M., Kojima-Kawagoe, M., Kondo, N., Shiba, T., Takamatsu, N., 2003. Comparative study of HP-27 gene promoter activities between the chipmunk and tree squirrel. Gene 302, 193–199.

Perez-Suarez, G., Arevalo, F., Lopez-Caballero, E., Lopez-Luna, P., 1990. Seasonal variations in hematological values and heart weight in 2 small mammals, a mouse, *Apodemus sylvaticus*, and a vole, *Pitymys duodecimcostatus*. Acta Theriol. 35, 201–208.

Place, N.J., Kenagy, G.J., 2000. Seasonal changes in plasma testosterone and glucocorticosteroids in free-living male yellow-pine chipmunks and the response to capture and handling. J. Comp. Physiol. B 170, 245–251.

Price, K., Boutin, S., Ydenberg, R., 1990. Intensity of territorial defense in red squirrels—and experimental test of the asymmetric war of attrition. Behav. Ecol. Sociobiol. 27, 217–222.

Rosenmann, M., Ruiz, G., 1993. Seasonal changes of blood values in the Andean mouse *Abrothrix andinus*. Comp. Biochem. Physiol. A 105, 119–122.

Scelza, J., Knoll, J., 1982. Seasonal variation in various blood indices of the kangaroo rat, *Dipodomys panamintinus*. Comp. Biochem. Physiol. A 71, 237–241.

Schulte-Hostedde, A.I., Millar, J.S., 2002. 'Little chipmunk' syndrome? Male body size and dominance in captive Yellow-Pine chipmunks (*Tamias amoenus*). Ethology 108, 127–137.

Schulte-Hostedde, A.I., Millar, J.S., Gibbs, H.L., 2002. Female-biased sexual size dimorphism in the yellow-pine chipmunk (*Tamias amoenus*): sex-specific patterns of annual reproductive success and survival. Evolution 56, 2519–2529.

Seal, U.S., Mech, L.D., VanBallenberghe, V., 1975. Blood analyses of wolf pups and their ecological and metabolic interpretation. J. Mammal. 56, 64–75.

Southwood, T.R.E., 1977. Habitat, the template for ecological strategies? J. Anim. Ecol. 46, 337–366.

Steele, M.A., 1998. *Tamiasciurus hudsonicus*. Mamm. Species 586, 1–9.

Sutton, D.A., 1992. *Tamias amoenus*. Mamm. Species 390, 1–8.

Takamatsu, N., Ohba, K., Kondo, J., Kondo, N., Shiba, T., 1993. Hibernation-associated gene regulation of plasma proteins with a collagen-like domain in mammalian hibernators. Mol. Cell. Biol. 13, 1516–1521.

Takamatsu, N., Kojima, M., Taniyama, M., Ohba, K., Uematsu, T., Segawa, C., Tsutou, S., Watanabe, M., Kondo, J., Kondo, N., Shiba, T., 1997. Expression of multiple alpha-1 antitrypsin-like genes in hibernating species of the squirrel family. Gene 204, 127–132.

Thompson, D.C., 1977a. Diurnal and seasonal activity of the grey squirrel (*Sciurus carolinensis*). Can. J. Zool. 55, 1185–1189.

Thompson, D.C., 1977b. Reproductive behavior of the grey squirrel. Can. J. Zool. 55, 1176–1184.

Trumble, S.J., Castellini, M.A., 2002. Blood chemistry, hematology, and morphology of wild harbor seal pups in Alaska. J. Wildl. Manage. 66, 1197–1207.

Weber, D.K., Danielson, K., Wright, S., Foley, J.E., 2002. Hematology and serum biochemistry values of dusky-footed wood rat (*Neotoma fuscipes*). J. Wildl. Dis. 38, 576–582.

Wenberg, G.M., Holland, J.C., Sewell, J., 1973. Some aspects of the hematology and immunology of the hibernating and non-hibernating woodchuck (*Marmota monax*). Comp. Biochem. Physiol. A 46, 513–518.

Wilson, D.E., Reeder, D.M. (Eds.), 1993. Mammal Species of the World. Smithsonian Institution Press, Washington, DC.

Wilson, D.E., Ruff, S. (Eds.), 1999. The Smithsonian Book of North American Mammals. UBC Press, Vancouver, Canada.