Journal of the American Association for Laboratory Animal Science
Copyright 2011
by the American Association for Laboratory Animal Science

Vol 50, No 3
May 2011
Pages 374–377

Effects of Aging and Blood Contamination on the Urinary Protein–Creatinine Ratio in Captive Chimpanzees (*Pan troglodytes*)

Michael L Lammey,[1,*] John J Ely,[1] Tony Zavaskis,[1] Elaine Videan,[1] and Meg M Sleeper[2]

The initial goal of this study was to evaluate proteinuria by using the protein to creatinine (UPC) ratio of urine obtained by cystocentesis of healthy adult captive chimpanzees. Urine samples were collected by using ultrasound-guided cystocentesis from 125 (80 male, 45 female) captive chimpanzees. All samples were collected over a 17-mo time period (August 2008 to January 2010) during the animal's annual physical examination. Samples were assayed at a veterinary diagnostic laboratory. Results indicated that both age and blood contamination affect the UPC ratio and therefore alter the diagnostic utility of the UPC ratio in chimpanzees. In addition, this research establishes reference ranges by age for the UPC ratio in healthy adult chimpanzees. Chimps younger than the median age of 24.6 y have a median UPC ratio of 0.098 (range, 0 to 1.76), whereas older animals have a median UPC of 0.288 (range, 0 to 2.44). Our results likely will enable veterinarians working with chimpanzees to better evaluate their renal function.

Renal diseases in its various forms have been reported to occur in captive chimpanzees but at a relatively low prevalence.[14,19,33,35] However, renal failure is a frequent cause of morbidity and mortality in captive chimpanzee populations.[26] Reliable means for measuring renal function are invaluable tools. Urinalysis is the traditional initial diagnostic test used to evaluate overall renal function. Collection of urine for 24 h followed by protein-to-creatinine clearance testing is used in human medicine to determine the level of renal function and assess kidney status.[6] However, this test can be difficult to administer to captive chimpanzees because of its time requirements. A spot urine test for protein and creatinine can be used to estimate the amount of protein excreted.[5] The urine protein-to-creatinine ratio obtained from the spot urine test corresponds to the amount of protein (in grams) excreted in 24 h, making this measure more appropriate for use in chimpanzees. Due to the increasingly geriatric population of captive chimpanzees, aging effects on anatomic structures and physiologic parameters have been noted.[28,36,40] In humans, the aging process reduces kidney function, the glomerular filtration rate, and the creatinine clearance rate and significantly increases the risk level for cardiovascular disease.[4]

Several studies have addressed the analysis of urine in wild chimpanzees.[22,24,25,29] All of these studies used urinary 'dipstick' test strips with free-catch urine samples. Only one study has compared the specific gravities of urine from captive and wild chimpanzees;[3] all samples were obtained by free catch, and specific gravity evaluated with a refractometer. Kidney function and urinary creatinine clearance in captive chimpanzees have also been evaluated by using free-catch samples and a urinometer.[11] To the best of our knowledge, the current study is the first that deals specifically with the urinary protein–creatinine (UPC) ratio of samples collected from chimpanzees by cystocentesis. The hypothesis of this study is that both age and blood contamination (even at the trace level) can alter the UPC ratio. If true, these factors need to be assessed so that UPC ratios determined from cystocentesis samples can function as an effective diagnostic tool for screening and evaluating renal disease in captive chimpanzees. In addition, we studied the effects of age and sex on reference intervals for the UPC ratio of healthy captive chimpanzees. Furthermore, we here describe guidelines for appropriate urine collection from chimpanzees by cystocentesis.

Materials and Methods

All chimpanzees at our facility (Alamogordo Primate Facility, Holloman Air Force Base, NM) are maintained in same-sex group housing to comply with the NIH chimpanzee breeding moratorium; no research or breeding occurs at our facility. The facility and its program are fully AAALAC-accredited, and all procedures were IACUC-approved. Chimpanzees were fed a commercial primate diet (Monkey Diet Jumbo 5LR2, Purina Mills, St Louis, MO) and were maintained in accordance with the *Guide for the Care and Use of Animals*.[20] A maximum of 6 chimpanzees are maintained in an indoor den (180 ft^2, 9.5-ft high) with radiant heated floor and air conditioning. In addition, chimpanzees have 24-h access to an outdoor area (242 ft^2, 12-ft high) as well as with weekly access to a modular geodesic dome structure (802 ft^2; Primadome, Brandes Brothers Constructors, Bee Cave, TX). Each chimpanzee participates in enrichment programs and is observed every 2 h by experienced, AALAS-certified animal care technicians or clinical veterinarians. The enrichment program involves daily fruits and vegetables and bi-weekly forage opportunities; novelty items including blankets, magazines, and simulated termite mound feeders are provided also. All animals are observed for general health, activity levels, elimination, exercise tolerance, and recovery rates. The subjects for this study consisted of 125 (45 female, 80 male) adult chimpanzees (age: range, 11.6 to 50.1 y; mean, 26.7 y). The infectious

Received: 18 Oct 2010. Revision requested: 22 Nov 2010. Accepted: 02 Dec 2010.
[1]Alamogordo Primate Facility, Holloman Air Base; Alamogordo, New Mexico; [2]Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
[*]Corresponding author. Email: michael.lammey@crl.com

disease status of the study population was: hepatitis-B-infected, 1 chimp; hepatitis-C-infected, 21; HIV-infected, 13; coinfected (hepatitis C and HIV), 3; and noninfected, 87.

From August 2008 through January 2010, we collected urine samples by cystocentesis during annual physical examinations of chimpanzees under sedation (3.0 mg/kg tiletamine hydrochloride–zolazepam). Included were a physical examination, CBC, clinical chemistry, electrocardiography, abdominal ultrasonography, tuberculosis testing, dental prophylaxis, and blood pressure assessment. Blood pressure measurements, electrocardiography, pO_2, and core body temperature were recorded by using a physiologic monitor (Datascope Passport 2, Mahwah, NJ).

The process of obtaining urine by cystocentesis of a chimpanzee remains challenging due to the difficult anatomic location of the bladder. The chimpanzee bladder is located dorsal and caudal within the pelvic canal. The palpable cranial edge of the ilium served as a guideline for a starting point of needle insertion. Clippers were used to remove the hair from the abdomen, and the lower abdominal area was prepared aseptically. A 3.25-in. spinal needle was attached to a 10-mL syringe to access the bladder. The needle was inserted at a 45° angle to the abdomen, and urine was transferred into the syringe by negative pressure. The use of concurrent ultrasonography ensured that the bladder and other abdominal structures were visible and that urine could be collection safely. The ultrasound technique also provided valuable information about the bladder wall structure, amount of urine present within the bladder, and possible anomalies (for example, uroliths) within the bladder.

All urine samples were obtained from chimpanzees that appeared clinically healthy prior to annual physical examination. Only urine samples that were free of contaminates, including RBC, WBC, and bacteria, were used to establish reference values. All urine samples were evaluated for pH levels. Urinalysis was performed at a veterinary diagnostics laboratory (Antech Diagnostics, Southhaven, MS) by using an automated reflectance spectrophotometer (Clinitek Atlas Automated Urine Chemistry Analyzer, Siemens, Tarrytown, NJ) and standard reagent packs (Siemens). The UPC ratio was quantified at a veterinary diagnostics laboratory (Antech Diagnostics) by using a random- access spectrophotometry analyzer (Olympus AU5431, Beckman Coulter, Brea, CA) using standard reagents (MTP [Urinary/CSF Microprotein] and Creatinine Reagent, Beckman Coulter).

Statistical analysis. All statistical modeling and tests were conducted using commercially available software (version 11, SYSTAT Software, Richmond, CA). Statistical significance was defined as a P value less than 0.05. UPC ratios were examined for fit to normal (Gaussian) assumptions by using the omnibus Shapiro–Wilks statistic.[10] Data were normalized by using the Box–Cox power series of transformations,[8] with a maximum likelihood procedure to estimate the λ parameter at −0.50.[16] Transformed data underwent ANOVA by using focused comparisons.[34,37] Animals on medications were excluded from analysis. Because age and sex are determinants of chimpanzee and human health and disease, these variables were evaluated as probable covariates.[12,13] Age was measured by decade of life. Blood contamination was categorized as a trichotomy (none, 1 to 2, and 3). Reference intervals were estimated from healthy chimpanzees by using the robust method according to CLSI/IFCC C28-A3 guidelines[9,16] and MedCalc software.[31] Covariates that were statistically significant in the ANOVA were tested with the z* statistic to determine the need for separate reference intervals.[16] If z* exceeded the critical value of 3.0, data were partitioned into subgroups for separate reference intervals.[9,16]

Results

Results of the statistical analysis indicated that, although sex was not a significant predictor ($F_{1,91} = 0.064$, $P = 0.801$), both age ($F_{1,92} = 9.59$, $P = 0.003$) and blood contamination ($F_{2,92} = 14.12$, $P < 0.000$) were significant predictors of the UPC ratio in captive chimpanzees. This model explained 31.5% of the variance in UPC ratio. As expected, the mean UPC ratio increased significantly ($P < 0.05$) with age. The UPC for chimpanzees 30 y or older (mean UPC ratio, 195.6) was 3 times higher than that of animals 20 to 29 y of age (63.4), which was more than 100 times higher than that animals 10 to 19 y old (0.51). In addition, mean UPC ratios increased dramatically across categories of blood contamination, from no contamination (mean UPC, 0.12), to trace to moderate contamination (0.27), to high contamination (6.83; Figure 1). This result indicates that blood contamination, even at trace levels, is a serious problem that can confound the interpretation of UPC values for evaluating renal function. In addition, geriatric chimpanzees (30 y or older) were 3.4 times more likely to have blood contamination than were animals 20 to 29 y old and 18.8 times more likely than were chimpanzees 10 to 19 y in age. This trend approached but did not reach statistical significance ($G^2_4 = 9.13$, $P = 0.058$). Chimpanzees that had a UPC greater than 1.0 were evaluated further with other diagnostic tests for renal disease (data not shown).

Reference intervals for UPC ratio were estimated. The z* test for geriatric animals was highly significant compared with those for 10- to 19-y-olds (z* = 6.57) and 20- to 29-y-olds (z* = 6.19), whereas there was no difference between the 2 younger groups (z* = 0.76). After elimination of outliers, small sample sizes prohibited partitioning by age, because the sample size was too small even for the robust method .[9,18] Therefore, a single 95% reference interval (0, 1.266) was estimated for healthy nonmedicated adults (Figure 2). Median UPC ratios were approximately twice as high for geriatric chimpanzees (median UPC ratio, 0.303) than for nongeriatric animals (0.153). These results suggest a slow but progressive decline in kidney function with age. This reference interval will facilitate the clinical determination of kidney failure in chimpanzees of any age, by enabling comparison to the reference interval specified for healthy animals of the same age group. This interval is valid for all adult chimpanzees not on medications and with no blood (even trace blood) in the collected urine sample.

Discussion

As the challenges of caring for geriatric apes increase, management strategies will need to adjust to accommodate for physiologic health risks that may be associated with aging.[40] Chronic renal failure has been linked to increasing age in humans.[21] In addition, renal failure has been noted to be a significant cause of morbidity and mortality in captive chimpanzee populations.[26] Appropriate management and evaluation of renal disease is needed as the captive chimpanzee population becomes increasingly older. The UPC ratio is an important initial test that can help define renal disease or stability.

Chronic kidney disease is defined as kidney damage or decreased kidney function for 3 or more months.[30] Persistent proteinuria is the principle marker of kidney damage.[23] At our facility, an initial renal examination includes dipstick testing (Urispec 11-way, Henry Schein Melville, NY). Further evaluations include CBC, blood chemistry (blood urea nitrogen,

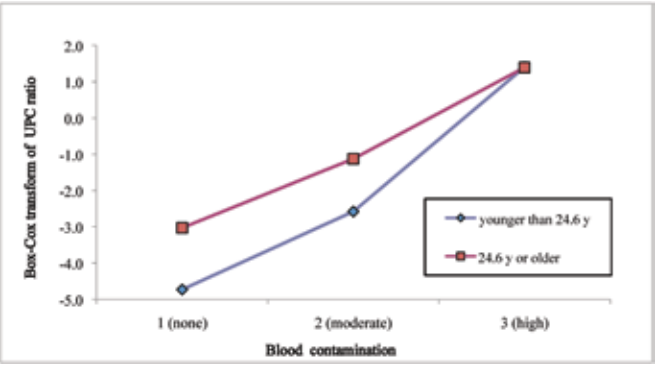

Figure 1. Effects of blood contamination on the UPC ratio.

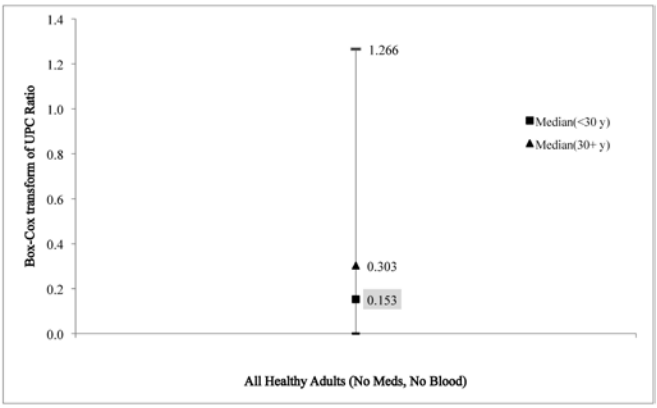

Figure 2. 95% reference interval and medians for UPC ratio.

creatinine, calcium, phosphate, and potassium), ultrasonography, and radiography. If chimps show any clinical signs of renal damage, they are monitored continuously for any sign of decompensation.

Cystocentesis has been a commonly used method of urine collection in veterinary medicine.[2] Other standard methods of urine collection in veterinary medicine are free catch and catheterization.[39] Iatrogenic introduction of hemorrhage is one of the disadvantages of obtaining urine samples by cystocentesis. Although cystocentesis can result in the contamination of a urine sample by RBC, it eliminates the bacterial contamination originating from the lower urinary tract that plagues most other collection methods.[32] The use of the cystocentesis method of urine collection reduces the bacteria that could contaminate a sample to falsely elevate the UPC ratio. By performing cystocentesis, secretions and debris of the lower urogenital tract are avoided, and interpretation of urinalysis findings is simplified.[39] Urine collection by cystocentesis is an appropriate method of obtaining a urine sample in chimpanzees when risks of bacterial contamination are high.

In veterinary medicine, blood contamination of urine samples may not cause an increase in UPC ratios.[38] However, our results indicated that even trace levels of blood contamination can confound the interpretation of estimated UPC ratios (Figure 1). Future studies should collect UPC data from a larger number of animals to decisively test this conjecture. Meanwhile, we recommend thorough evaluation of blood contamination of urine samples when interpreting UPC ratios as a diagnostic method to assess kidney function.

Appropriately measuring the level of proteinuria is vital to evaluating protein loss and therefore kidney function. Proteinuria is a sensitive indicator of renal damage.[32] The magnitude of proteinuria should be measured accurately and precisely by using quantitative tests.[15] In domestic species such as dogs, a UPC ratio less than 0.5 is considered normal, and a UPC ratio greater than 1.0 indicates proteinuria.[15] In human medicine, a UPC ratio of 0.1 is considered normal, whereas a UPC ratio greater than 2 is usually associated with glomerulopathy producing the nephrotic syndrome.[7] Our current research indicates that captive chimpanzees have a normal UPC ratio (0.288) that is similar to that of humans.

Nephrotic syndrome is a glomerular disease that is characterized by the presence of proteinuria, hypoalbuminemia, edema, hyperlipuria, and hyperlipemia.[1] The leading diagnostic feature of renal glomerular diseases is proteinuria.[17] The finding of proteinuria of greater than 3.5 g/24 h/1.73 m^2 is sufficient for the designation of nephrotic syndrome.[1] In humans, proteinuria greater than 3.5 g in 24 h indicates glomerular disease.[5] Nephrotic syndrome has been described in captive chimpanzees.[27] The UPC values presented in this study can provide valuable guidelines for diagnosing and evaluating chimpanzees with nephrotic syndrome.

Renal disease has been noted in various captive chimpanzees in the past.[14,19,33,35] However, little is known about renal failure in general in this species or about how to manage this disease. Urinalysis and a UPC ratio is the initial testing needed to evaluate renal function. As the captive chimpanzee population continues to age, increased research into the various methods and tests to evaluate various disorders associated with aging is needed. Further studies to increase our knowledge of renal dysfunction in chimpanzees are warranted.

Acknowledgments

This study was supported by NIH contract no. NO2-RR-1-209. We thank Barbara Davis (Antech Laboratories) for her help and support.

References

1. **Abul-Ezz SR, Bunke CM, Singh H, Shah SV.** 1997. Glomerular diseases, p 209–216. In: Andreoli TE, Carpenter CJ, Bennett JC, Plum F, editors. Cecil's essentials of medicine, 4th ed. Philadelphia (PA): WB Saunders.
2. **Adams RJ.** 2002. Techniques of experimentation, p 1019–1021. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, 2nd ed. New York (NY): Academic Press.
3. **Anestis SF, Breakey AA, Beuerlein MM, Bribiescas RG.** 2009. Specific gravity as an alternative to creatinine for estimating urine concentration in captive and wild chimpanzee (*Pan troglodytes*) samples. Am J Primatol **71:**130–135.
4. **Bai X, Han L, Liu J, Zhang W, Zhou H, Dong S, Sun Y, Chen X.** 2010. The relationship between age-related kidney dysfunction and Framingham risk score in healthy people in China. Curr Aging Sci **3:**188–197.
5. **Bazari H.** 2004. Approach to the patient with renal disease, p.654–662. In: Goldman L, Ausiello D, editors. Cecil's textbook of medicine, 22nd ed. Philadelphia (PA): WB Saunders.
6. **Benz RL.** 1996. Chronic renal failure, p 538–539. In: Rakel RE, editor. Saunders' manual of medical practice. Philadelphia (PA): WB Saunders.
7. **Berkow R, editor.** 1992. The glomerular diseases, p 1684–1702. The Merck manual of diagnosis and therapy, 16th ed. Rahway (NJ): Merck Research Laboratories.
8. **Box G, Cox D.** 1964. An analysis of transformations. J R Stat Soc Series B Stat Methodol **26:**211–252.
9. **Clinical and Laboratory Standards Institute.** 2008. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guidelines—3rd ed. CLSI document C28-A3. Wayne (PA): Clinical and Laboratory Standards Institute.
10. **Conover WJ.** 1980. Practical nonparametric statistics. New York (NY): Wiley.

11. **Eder G.** 1996. A longitudinal study of the kidney functions of the chimpanzee (*Pan troglodytes*) in comparison with humans. Eur J Clin Chem Clin Biochem **34:**889–896.
12. **Ely JJ, Bishop MA, Lammey ML, Sleeper MM, Steiner JM, Lee DR.** 2010. Use of biomarkers of collagen types I and II fibrosis metabolism to detect chimpanzee cardiovascular and renal disease. Comp Med **60:**154–158.
13. **Friis RH, Sellers TA.** 2009. Epidemiology for public health practice, 4th ed. Sudbury (MA): Jones and Bartlett Publishers.
14. **Greenwood AG, Lowe JW, Gaunt L.** 1995. Renal carcinoma in a chimpanzee (*Pan troglodytes*). Vet Rec **137:**380–381.
15. **Gregory CR.** 2003. Urinary system, p 231–259. In: Latimer KS, Mahaffey EA, Prasse KW, editors. Duncan and Prasses's veterinary laboratory medicine: clinical pathology, 4th ed. Ames (IA): Iowa State Press.
16. **Harris EK, Boyd JC.** 1995. Statistical basis of reference values in laboratory medicine. New York (NY): Marcel Dekker.
17. **Hildebrandt F.** 2010. Genetic kidney diseases. Lancet **375:** 1287–1295.
18. **Horn PS, Pesce AJ, Copeland BE.** 1998. A robust approach to reference interval estimation and evaluation. Clin Chem **44:**622–631.
19. **Hubbard GB, Lee DR, Eichberg JW.** 1991. Diseases and pathology of chimpanzees at the Southwest Foundation for Biomedical Research. Am J Primatol **24:**273–282.
20. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
21. **Jungers P, Chauveau P, Descamps-Latscha B, Labrunie M, Giraud E, Man NK, Grunfeld JP, Jacobs C.** 1996. Age- and gender-related incidence of chronic renal failure in a French urban area: a prospective epidemiologic study. Nephrol Dial Transplant **11:**1542–1546.
22. **Kaur T, Huffman MA.** 2004. Descriptive urological record of chimpanzees (*Pan troglodytes*) in the wild and limitations associated with using multireagent dipstick test strips. J Med Primatol **33:**187–196.
23. **Keane WF, Eknoyan G.** 1999. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE): a position paper of the National Kidney Foundation. Am J Kidney Dis **33:**1004–1010.
24. **Kelly TR, Sleeman JM, Wrangham R.** 2004. Urinalysis in free-living chimpanzees (*Pan troglodytes schweinfurthii*) in Uganda. Vet Rec **154:**729–730.
25. **Krief, S, Huffman MA, Sevenet T, Guillot J, Bories, Hladik CM, Wrangham RW.** 2005. Noninvasive monitoring of the health of *Pan troglogytes schweinfurthii* in the Kibale National Park, Uganda. Int J Primatol **26**.467–490.
26. **Lammey ML, Baskin GB, Gigliotti A, Lee DR, Ely JJ, Sleeper MM.** 2008. Interstitial myocardial fibrosis in a captive chimpanzee (*Pan troglodytes*) population. Comp Med **58:**389–391.
27. **Lammey ML, Lee DR.** 2008. Diagnosis and treatment of nephrotic syndrome in a male captive chimpanzee (*Pan troglodytes*). J Am Assoc Lab Anim Sci **47:**57.
28. **Lammey ML, Lee DR, Ely JJ, Sleeper MM.** 2008. Sudden cardiac death in 13 captive chimpanzees. J Med Primatol **37 Suppl 1:**39–43.
29. **Leendertz SAJ, Metzger S, Skjerve E, Deschner T, Boesch C, Riedel J, Leendertz FH.** 2010. A longitudinal study of urinary dipstick parameters in wild chimpanzees (*Pan troglodytes verus*) in Cote d'Ivorie. Am J Primatol **72:**689–698.
30. **Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes AW, Hogg RJ, Perrone RD, Lau J, Eknoyan G.** 2003. National Kidney Foundation practice guideline for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med **139:**137–147.
31. **MedCalc Software.** [Internet]. 2010. MedCalc Software, version 11.3. [Cited 3 June 2010]. Available at: http://www.medcalc.be/download.php
32. **Ragen HA.** 1989. Markers of renal function and injury, p 321–343. In: Loeb WF, Quimby FW, editors. The clinical chemistry of laboratory animals. New York (NY): Pergamon Press.
33. **Rosenblum IY, Coulston F.** 1983. Impaired renal function in diabetic chimpanzees (*Pan troglodytes*). Exp Mol Pathol **38:**224–229.
34. **Rosenthal R, Rosnow RL.** 1985. Contrast analysis. Cambridge (UK): Cambridge University Press.
35. **Schmidt RE, Butler TM.** 1973. Glomerulonephritis and uremia in a chimpanzee. J Med Primatol **2:**144–154.
36. **Sleeper MM, Doane CJ, Langner PH, Curtis SK, Avila K, Lee DR.** 2005. Successful treatment of idiopathic dilated cardiomyopathy in an adult chimpanzee (*Pan troglodytes*). Comp Med **55:**80–84.
37. **Snedecor GW, Cochrane WG.** 1967. Statistical methods, 6th ed. Ames (IA): Iowa State University Press.
38. **Vaden SL, Pressler BM, Lappin MR, Jensen WA.** 2004. Effects of urinary tract inflammation and sample blood contamination on urine albumin and total protein concentrations in canine urine samples. Vet Clin Pathol **33:**14–19.
39. **Van Steenhouse JL.** 2002. Clinical pathology, p 152–157. In: McCurnin DM, Bassert JM, editors. Clinical textbook for veterinary technicians, 5th ed. Philadelphia (PA): WB Saunders.
40. **Videan EN, Fritz J, Murphy J.** 2008. Effects of aging on hematology and serum clinical chemistry in chimpanzee *(Pan Troglodytes*). Am J Primatol **70:**327–338.