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SHORT COMMUNICATION

FLUORESCENCE PROVIDES EVIDENCE OF CONGENITAL ERYTHROPOIETIC PORPHYRIA IN 7000-YEAR-OLD SPECIMENS OF THE EASTERN FOX SQUIRREL (*SCIURUS NIGER*) FROM THE DEVIL'S DEN

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Numerous fossil vertebrates, including the eastern fox squirrel (*Sciurus niger*), have been recovered from the Devil's Den Sinkhole in Levy County, Florida. The majority of the fossils have been recovered from a lateral passage ('Chamber 3') that lies approximately 70 feet below the current water surface (Martin and Webb, 1974). Fossils have been recovered from both an upper surface layer and an underlying yellow layer. Both of these units have produced extinct taxa (*Tremarctos floridanus*, *Platygonus compressus*, *Megalonyx* sp.), although remains from extinct taxa are more common in the yellow layer (Martin and Webb, 1974). The age of these deposits has been reported as early Holocene (approximately 7000–8000 years before present [ybp]) (Martin and Webb, 1974; Holman, 1978).

Both layers in Chamber 3 have produced remains of *S. niger*, including cranial, mandibular, limb, and girdle elements. The majority of these remains are blackened and mineralized. A small minority are light brown, and they appear to have undergone less mineralization. The amount of mineralization does not seem to correlate directly with age or with stratigraphic position; both types of preservation are found in both the surface and yellow layers. Moreover, some limb elements of *Sciurus* exhibit both types of preservation on the same bone, suggesting that the different preservational styles are the result of small-scale differences in diagenetic histories rather than different ages.

Modern populations of the eastern fox squirrel include individuals that display physiological peculiarities related to heme production. Heme is an important component of hemoglobin, the red pigment in vertebrate blood, which allows effective delivery of oxygen to tissues. The heme biosynthetic pathway consists of eight enzymes that sequentially convert glycine and succinyl coenzyme A (CoA) to heme (van Tuyll van Serooskerken et al., 2010). Mutations in the genes that produce these enzymes lead to a class of debilitating diseases called porphyrias, including congenital erythropoietic porphyria (CEP), which is also known as Günther's disease when it affects humans (Richard et al., 2008). Symptoms of CEP include anemia, cutaneous photosensitivity, and/or acute neurological attacks (van Tuyll van Serooskerken et al., 2010). Individuals with CEP accumulate excess uroporphyrin I in their bones, causing them to fluoresce under ultraviolet (UV) light (Turner, 1937; Flyger and Levin, 1977).

CEP has been documented in at least eight mammalian species besides humans (Richard et al., 2008; Rivera and Leung, 2008; van Tuyll van Serooskerken et al., 2010), but these are spontaneous occurrences except in the eastern fox squirrel (Turner, 1937; Flyger and Levin, 1977). In *S. niger* this condition has been reported in modern individuals from widely separated localities, including Pennsylvania, Michigan, Maryland, Texas, and Oklahoma (Turner, 1937; Allen, 1943; Levin and Flyger, 1971, 1973; Spradling et al., 2000). Indeed, Flyger and Levin (1977) promoted the use of the eastern fox squirrel as an inexpensive laboratory model for experimental studies of this disease. Laboratory investigations that described physiological details of porphyria in live *S. niger* included representatives of the closely related eastern gray squirrel, *S. carolinensis,* as controls (Levin and Flyger, 1973). Levin and Flyger (1973) reported high concentrations of uroporphyrin I in the bones, teeth, blood, soft tissues, and urine of eastern fox squirrels, but not eastern gray squirrels, which they characterized as being nonporphyric. Eastern fox squirrels do not exhibit the debilitating symptoms of CEP, and the persistence of CEP in *S. niger* indicates that the mutation causing this condition is either harmless or advantageous in this species (Turner, 1937; Levin and Flyger, 1971, 1973). Levin and Flyger (1971) and Flyger and Levin (1977) suggested that the mutation causing CEP in *S. niger* arose either in an ancestral population of the eastern fox squirrel or, perhaps, even before this species split from other tree squirrels.

Although Pleistocene and early Holocene sciurid remains are not uncommon from sites throughout eastern North America, only a small number of these remains have been referred to *S. niger*. This is likely due, in part, to the conservative nature of the skeletal morphology of tree squirrels (Emry and Thorington, 1982) and concomitant morphologic similarities between *S. niger* and *S. carolinensis*. In addition to similarities in their morphology, several different types of molecular genetic evidence, including serum albumin (Ellis and Maxson 1980), metabolic enzymes (Hafner et al., 1994), and sequences of the mitochondrial DNA (mtDNA) cytochrome *b* gene (Oshida and Masuda, 2000; Moncrief et al., 2010), indicate that *S. niger* and *S. carolinensis* are more closely related to each other than either is to other members of the genus.

Modern populations of these two species are sympatric over most of eastern North America; both species currently occur in temperate forests east of the Rocky Mountains (Koprowski, 1994a, 1994b). Although *S. niger* is often larger than *S. carolinensis*, there is an overlap in the size ranges in modern specimens of these species. Body mass of adult *S. niger* ranges from about 500 to about 1300 g, whereas adult *S. carolinensis* range in body mass from about 300 to about 700 g (Koprowski, 1994a, 1994b). All these factors complicate, or prevent, definitive identification of fossil remains unless very specific elements are preserved, or unless the *S. niger* elements are from particularly large individuals.

As part of a larger study of Pleistocene biogeography in these two species, we seek a method to identify fossils of *S. niger* and *S. carolinensis* easily and definitively in mixed assemblages. After learning that excess uroporphyrin I causes the bones of living *S. niger* to fluoresce under UV light, we speculated that fossils of this species might also exhibit this property. We chose a series of specimens from Devils' Den because they clearly are referable to *S. niger* based on the size of the elements. This locality also includes specimens that clearly are referable to *S. carolinensis* and *Tamias* sp., another sciurid. Therefore this locality also provided a series of controls for our investigations. The purpose of this study was to test fluorescence in fossils of *S. niger* in addition to other sciuruids from the same assemblage.

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