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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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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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METHODS

We examined collections from Devil's Den housed at the Florida Museum of Natural History (UF) for fluorescence under a UVP Model UVL-56 ultraviolet lamp, emitting at 366 nm at 6 watts. Specimens examined included both mineralized and nonmineralized elements from *S. niger*, as well as nonmineralized elements from *S. carolinensis* and *Tamias* sp. The Devil's Den specimens were compared to a modern specimen of *S. niger* from the Virginia Museum of Natural History (VMNH 33876 [VMNH 456 under old catalog], an adult female, collected 5 October 1990, 5 miles (8 km) north of Churchville, Augusta County, Virginia) for similarities in morphology and fluorescence patterns.

RESULTS

None of the blackened, mineralized remains fluoresced. However, we observed fluorescence in several of the light brown specimens of *S. niger* (2 of 2 crania, 1 of 2 dentaries, and 1 of 7 long bones; Fig. 1, Table 1). We did not observe fluorescence in any of the elements from other sciurids from Devil's Den exhibiting the light brown preservation (Table 1). Although the fluorescence in the Devil's Den specimens of *S. niger* was neither as pronounced nor as extensive as in the modern specimen, fluorescence was generally observed in the same regions of the

TABLE 1. Nonmineralized sciurid specimens examined in this study, and their response to ultraviolet light.

Catalog number	Taxon	Element	Fluorescence
UF 3608a	*Sciurus niger*	Cranium	Fluorescent
UF 3611	*Sciurus niger*	Fragmented skull	Fluorescent
UF 49519	*Sciurus niger*	Right dentary	Fluorescent
UF 49516	*Sciurus niger*	Left dentary	Non-fluorescent
UF 49749	*Sciurus niger*	Humerus	Fluorescent
UF 50010	*Sciurus niger*	3 tibiae	Non-fluorescent
UF 55294	*Sciurus niger*	2 humeri	Non-fluorescent
UF 55295	*Sciurus niger*	Humerus	Non-fluorescent
UF 13428	*Sciurus carolinensis*	Femur	Non-fluorescent
UF 13448	*Sciurus carolinensis*	Right dentary	Non-fluorescent
UF 55291	?*Sciurus carolinensis*	Femur	Non-fluorescent
UF 3612	*Tamias* sp.	Left dentary	Non-fluorescent
UF 17389	*Tamias* sp.	Left dentary	Non-fluorescent

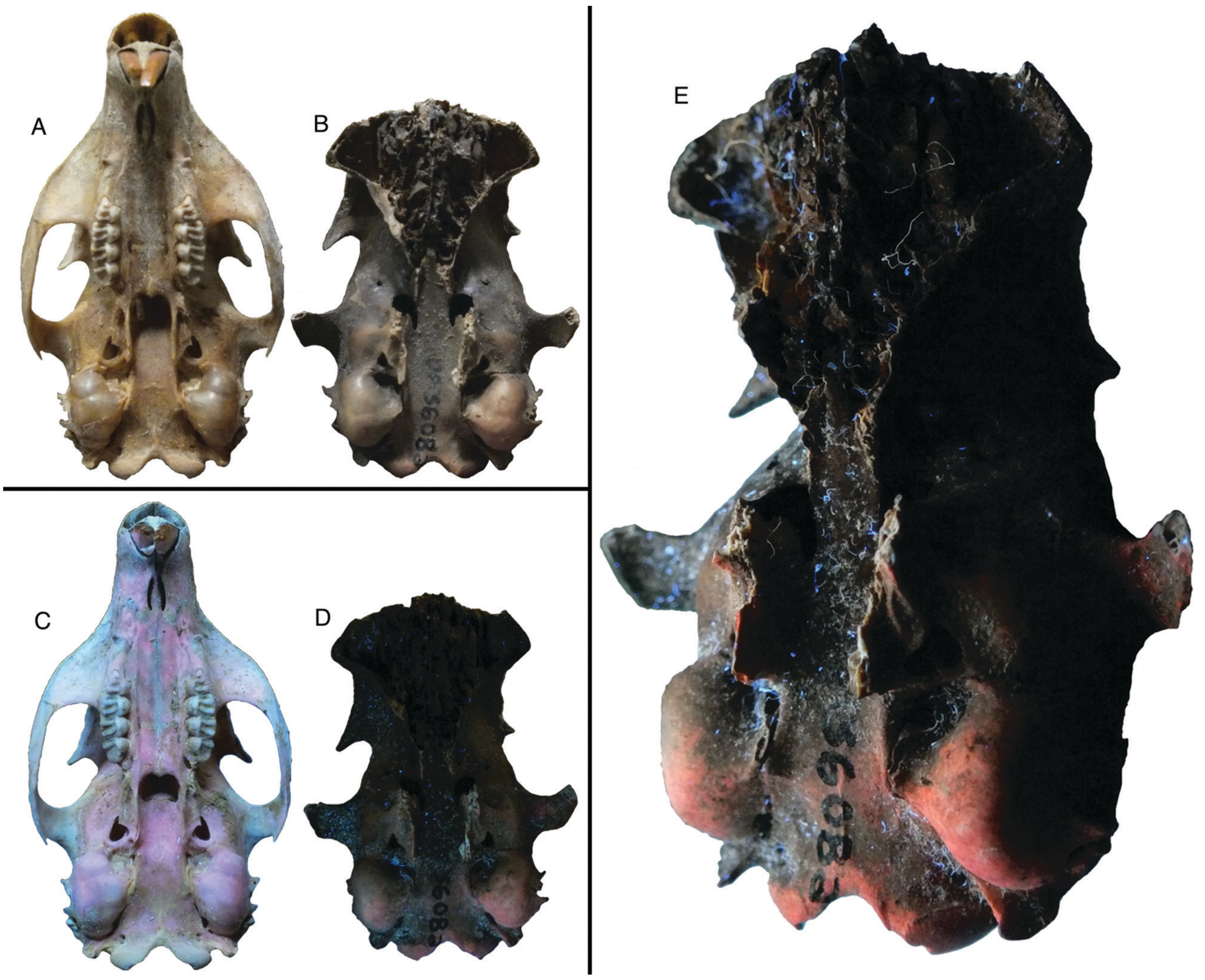

FIGURE 1. Crania of modern and subfossil *Sciurus niger* under white light and ultraviolet light. **A**, **C**, modern specimen (VMNH 456) and **B**, **D**, subfossil specimen from Devil's Den Sinkhole (UF3608a), ventral view. **A** and **B** were photographed under white light, **C** and **D** were photographed under ultraviolet light. **E**, closeup of UF3608a in ventrolateral view, taken under ultraviolet light.

elements. We observed the strongest fluorescence in the crania (the tympanic bullae, basicranium, and occipital condyles; Fig. 1C, D). Weaker fluorescence was observed on the maxillae, frontals, and dentary. We also observed fluorescence in a single fossil humerus as well as in the long bones of the modern specimen; however, the fluorescent signal in both cases was diffuse and patchy.

DISCUSSION

These results are evidence of CEP in at least some specimens of *S. niger* at least 7000 years ago. Because CEP is found in *S. niger*, but not *S. carolinensis*, this method provides an inexpensive, nondestructive test that can distinguish between these two species in mixed fossil assemblages. Even though the CEP signature apparently degrades over time, is subject to loss under certain preservational conditions, and is most prominent in certain bones, we maintain that the occurrence of fluorescent bones in a mixed assemblage of sciurid remains can be used as an initial and rapid test for the presence of *S. niger*, which is necessary to conduct comparative studies of Pleistocene biogeography in sciurids.

Levin and Flyger (1971) and Flyger and Levin (1977) demonstrated that CEP exists in widely separated, naturally occurring modern populations of *S. niger* (from the states of Maryland and Texas) and suggested that CEP is universal in *S. niger*. However, a more recent study (Spradling et al., 2000) reported fluorescence in only about 70% of individuals in some western populations (from the adjacent states of Oklahoma and Texas). Spradling et al. (2000) raised the possibility that curatorial treatments for cleaning modern skeletal material might have negatively affected the fluorescent properties of some of the specimens they examined. They performed a test in which they subjected a modern specimen to various concentrations of cleaning solutions. They discounted this as an explanation for the fact that they observed less than 100% fluorescence. In the end, Spradling et al. (2000) concluded that bone fluorescence (and, by inference, CEP) is a polymorphic character in eastern fox squirrels. We suggest that additional studies should be performed to assess the effect of different cleaning methods on bone fluorescence. We also agree with Spradling et al. (2000) that fluorescence in modern specimens from throughout the range should be investigated systematically.

Regardless of the frequency at which it occurs in modern populations of *S. niger*, the presence of porphyria in early Holocene specimens and the lack of ill effects from CEP in modern individuals suggest that CEP in the eastern fox squirrel is not the result of multiple spontaneous mutations in numerous populations. Instead, we suggest that it is the ancestral condition in this species. We also suggest that this trait can be used to investigate the biogeographic history of *S. niger* because it allows, for the first time, definitive identification of this taxon in deposits that include similar-sized remains of *S. carolinensis*.

A recent study of the evolutionary history of eastern fox squirrels (Moncrief et al., 2010) used evidence from a neutral genetic marker (a 402-base-pair segment of the mtDNA cytochrome *b* gene) to conclude that *S. niger* experienced range contraction into one or more refugia during the Pleistocene, followed by rapid post-glacial range expansion within the past 14,000 years. Moncrief et al. (2010) were not able to infer the location of the Pleistocene refugia using mtDNA evidence. Further investigation of the CEP marker (which is a nuclear mutation) in both fossil and modern populations of *S. niger* may provide the evidence necessary for such inference. Such studies should address, among other topics, questions regarding the frequency at which this trait occurs in populations of eastern fox squirrels across the entire geographic and temporal range of the species.

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