

MITOCHONDRIAL DNA AND METEOROLOGICAL DATA SUGGEST A CARIBBEAN ORIGIN FOR NEW MEXICO'S FIRST SOOTY TERN

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ABSTRACT: We report the first documented record for the Sooty Tern (*Onychoprion fuscatus*) in New Mexico and the fourth for the region of the southern Rocky Mountains and trans-Pecos Texas. The bird was found dead in moderately fresh condition on 8 July 2010 in the Laguna Grande area, near Carlsbad, Eddy County. It was brought to the Museum of Southwestern Biology where it was preserved as a study skin. A DNA analysis comparing the sequence of the specimen's mitochondrial control region to a published population-genetic dataset on this species found that the sequence of the New Mexico tern was a perfect match with previously sequenced haplotypes from Puerto Rico and Ascension Island and ~2% divergent on average from all Sooty Terns previously sequenced from the Pacific and Indian oceans. Measurements of the specimen are consistent with a Caribbean origin. We surmise that this individual was carried inland from the Gulf of Mexico to southeastern New Mexico by the remnants of Hurricane Alex.

The Sooty Tern (*Onychoprion fuscatus*) is a seabird that nests on tropical and subtropical islands worldwide (Schreiber et al. 2002). Although the species typically remains at sea, it is known to wander widely, often in association with tropical storms (e.g., Dickerman et al. 1998, Hockey et al. 2005, Robin and Sudheendra Rao 2005). In this paper, we describe a Sooty Tern specimen that was salvaged far inland in the southwestern United States and that represents one of only a few records for the region. We attempt to identify the natal origins of this specimen by comparing its mitochondrial DNA and measurements to those of Sooty Terns from potential source populations in the central Pacific Ocean, the eastern tropical Pacific Ocean, the Caribbean Sea, and the central Atlantic Ocean. We also consider weather that may have driven this bird inland.

On 8 July 2010, staff of the Mosaic Corporation discovered a dead tern in a brine pond in the Laguna Grande area southeast of Carlsbad, New Mexico. The specimen was transferred to Desert Willow Veterinary Services where it was tentatively identified as a Sooty Tern and shipped to the Museum of Southwestern Biology (MSB), University of New Mexico. Johnson prepared it as a study skin (MSB 30000; <http://arctos.database.museum/guid/MSB:Bird:30000>; Figure 1). Its identification was confirmed by comparison to another Sooty Tern skin at MSB. The specimen was received soaked in water and covered with debris but was fresh enough that it could be preserved as a study skin with minimal loss of feathers. Its internal anatomy was still intact. Its ovary was 8 × 4 mm, with no developing ova, oviduct rather straight, 3 1/2 mm wide; the bursa of Fabricius was absent. It was molting primary 3 and rectrices 1 and 6; on the head and neck molt was moderate, on the rest of the body light. Its plumage in general was rather worn and faded. Freshly grown feathers on the back were edged in

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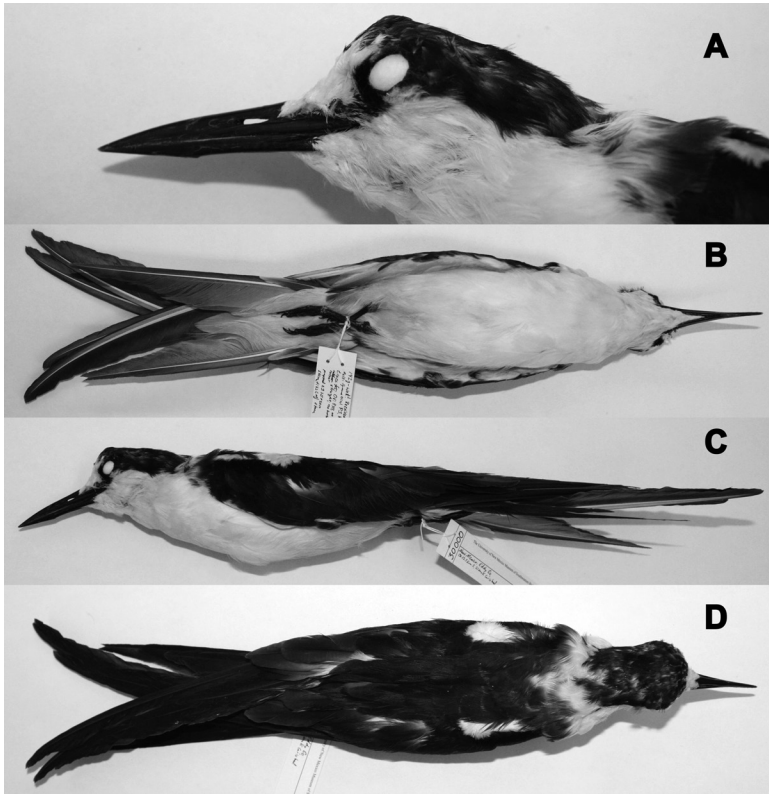


Figure 1. Photographs of the New Mexico specimen of the Sooty Tern, MSB 30000; (A) close up of head; (B) ventral view; (C) lateral view; (D) dorsal view.

white. During preparation, many feathers on the dorsal surface of the base of the neck were lost, resulting in the specimen having a capped appearance, rather than an entirely black dorsum as it originally had. See Table 1 for comparison of the specimen's tarsus, exposed culmen, and wing chord measurements to those of various populations of the Sooty Tern.

In the Atlantic basin the breeding range of the Sooty Tern comprises coastal islets of the Gulf of Mexico. Although small nesting populations exist in Louisiana and western Florida, the bulk of the population breeds in the Yucatán, and on Caribbean islands from the Dry Tortugas and the Bahamas through the West Indies, Fernando de Noronha, Ascension, Martin Vas, and St. Helena Islands (AOU 1957). In the central and eastern Pacific Ocean, it breeds from the Hawaiian Islands to Islas Revillagigedo and Tres Mariás along the western coast of Mexico (AOU 1957) and south to subtropical Chilean islands (e.g., Easter Island; Schreiber et al. 2002). Most authorities recognize seven subspecies of Sooty Tern, three of which are known from the waters of the United States, Mexico, and Central America (Schreiber et

Table 1 Comparison of Measurements of the Sooty and Bridled Terns

Locality	Ocean	Sex	n	Wing	Culmen	Tarsus	Source
Sooty Tern							
New Mexico (MSB 30000)	—	F	1	277	39.0	21.8	
Johnston Atoll, Hawaii	Pacific	F	16	291 ± 8	43.0 ± 1.6	24.5 ± 1.8	LACM ^a
Johnston Atoll, Hawaii (2002)	Pacific	U	59	295 ± 7	44.0 ± 1.7	29.7 ± 0.9	Schreiber et al.
Nayarit, Mexico	Pacific	F	7	286 ± 6	40.5 ± 2.2	21.3 ± 0.4	LACM
Cocos Island	Pacific	F	2	285 ± 0	41.2 ± 2.9	22.0 ± 1.4	LACM
Clipperton Island	Pacific	F	2	285 ± 5	42.4 ± 1.1	21.9 ± 0.5	LACM
Christmas Island (2002)	Indian	U	117	286 ± 7	43.0 ± 1.7	28.8 ± 1.1	Schreiber et al.
Ascension Island	Atlantic	U	≥986	293 ± 8	43.5 ± 2	—	Hughes et al. (2010)
Atol das Rocas, Brazil	Atlantic	U	50	289 ± 6	43.2 ± 1.8	—	Schulz-Neto (1998)
Tinhosa Grande, São Tomé (1997);	Atlantic	U	≥28	284 ± 9	41.0 ± 1.7	—	Monteiro et al.
Dry Tortugas, Florida (2002)	Atlantic	U	50	289 ± 7	44.0 ± 1.8	28.9 ± 1.1	Jones and Tye (2006) Schreiber et al.
Florida (mixed localities)	Atlantic	F	2	281 ± 1	43.3 ± 2.9	22.9 ± 2.1	LACM, MSB
Bridled Tern (off North Carolina)	Atlantic	F	≥16	257.2 ± 23.6	38.0 ± 1.4	24.5 ± 2.6	Haney et al. (1999)

^aLACM, specimens in Natural History Museum of Los Angeles County, Los Angeles.

al. 2002, Dickinson 2003). We consider three as candidates for vagrancy to New Mexico: *Onychoprion f. fuscatus* (Linnaeus), *O. f. crissalis* (Lawrence), and *O. f. oahuensis* (Bloxham). The eastern Pacific *O. f. crissalis* has the underparts grayer than in the western Atlantic *O. f. fuscatus*. The central Pacific *O. f. oahuensis* is similar in color to *O. f. crissalis*, but its bill averages larger (Cramp 1985). Available data suggest that there is little geographic variation in size throughout the range of the Sooty Tern, although North Pacific populations appear to be larger in body mass, wing length, and tarsus length (Schreiber et al. 2002).

On a global scale, the Sooty Tern has slight mitochondrial genetic differentiation, which is thought to have arisen after global expansion during the last ~100,000 years (Avise et al. 2000, Peck and Congdon 2004). Avise et al. (2000) sequenced a 373-base-pair (bp) portion of part I of the mitochondrial control region of Sooty Terns from the Caribbean and tropical Atlantic, central tropical Pacific, and central and western Indian Ocean. They found evidence for a dichotomous split between the Atlantic and Indo-Pacific populations, with a net genetic distance of ~1.5% between the two groups. Peck and Congdon (2004) were unsuccessful in sequencing additional samples for part I of the control region, but they added sequences of ~540 bp of part II of the control region of Sooty Terns from three separate breeding populations in the Great Barrier Reef region of the southwest Pacific Ocean, and they reanalyzed the data of Avise et al. (2000). They found that all Sooty Tern populations that had been assayed showed evidence of population bottlenecks and subsequent expansions that are estimated to have occurred between 16,000 and 90,000 years ago. To date, no study has sequenced Sooty Terns from subspecies *crissalis* of the eastern tropical Pacific.

METHODS

Using Qiagen DNEasy kits, we extracted DNA from 0.25 mg of muscle tissue and from the proximal tip of a single axillary feather of our specimen by following the manufacturer's protocol but with the addition of 30 μ L of 0.1-M dithiothreitol to the feather fragment at the initial tissue-incubation and digestion phases to reduce the disulfide bonds of the keratinous rachis and calamus. We assayed both extractions for DNA content with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA). Following Avise et al. (2000) and using the primers they reported, we attempted to amplify a 343-bp fragment of the mitochondrial control region.

However, the primer combination reportedly used by Avise et al. (2000) resulted in amplification and sequencing of a pseudogene that was not alignable with the published sequences for the Sooty Tern's control region. We subsequently tried without success to amplify the target fragment by using three additional primer pairs that have been used for sequencing the control region in other species of Charadriiformes. The success of Avise et al. with these primers may have resulted from their use of purified mitochondria rather than whole DNA extract that contains a mix of mitochondrial and nuclear DNA. Finally, we designed primers directly from the Avise et al. (2000) sequence (Genbank accession no. AF205605.1) that would amplify a 271-bp fragment between the 5' forward primer (Avise 325F: GTATTA-

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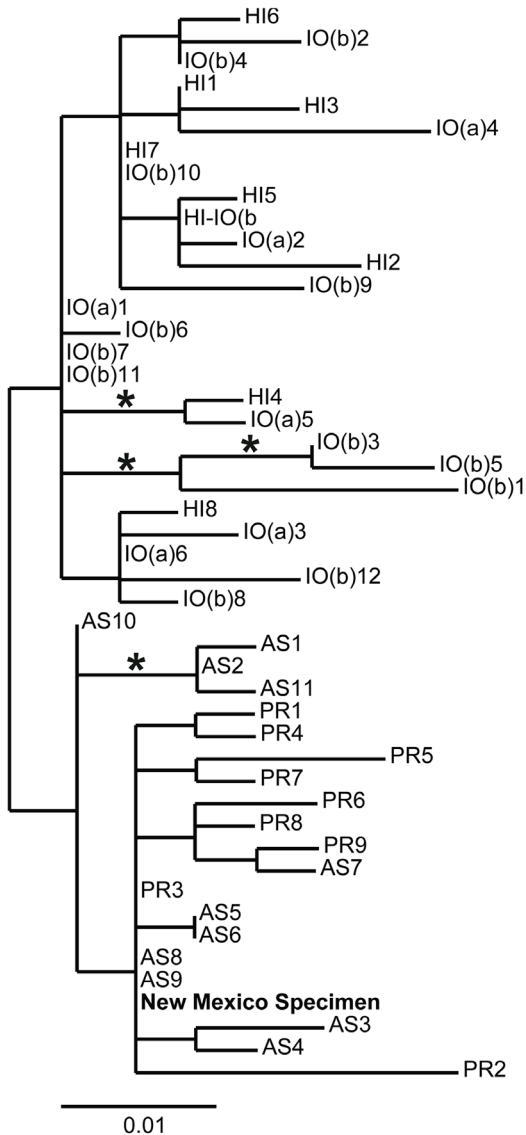


Figure 2. Phylogeny of Sooty Tern mitochondrial haplotypes based on 271 bp of the control region. Nodes supported by >70% bootstrap support are marked with asterisks and correspond to the haplotypes published by Avise et al. (2000). IO(a), Chagos Archipelago, Indian Ocean; IO(b), Seychelles, Indian Ocean; HI, Johnston Atoll (near Hawaii), Pacific Ocean; PR, Puerto Rico, Atlantic Ocean; AS, Ascension Island, Atlantic Ocean. Note the position of the New Mexico specimen in a clade of samples from Puerto Rico and Ascension Island.

CATACAATAATATCCCCCATT) and the 3' reverse primer (Avise325R: ACGATTAATAAATCCATCTAATACGAA). We amplified this fragment in a 15- μ L reaction using 2 μ L of the DNA extract and the following reagents: 0.15 μ L of Taq polymerase (0.75 units; Gold Taq, ABI, Mountain View, CA), 200 μ M of each deoxyribonucleotide triphosphate, 1.5-mM MgCl₂, 1.5 μ L Gold Buffer (ABI), and 0.5 μ M of each primer. For the polymerase chain reaction (PCR), we used an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) thermal cycler to carry out the following protocol: 95 °C for 8 min, (95 °C for 30 sec, 50 °C for 30 sec, 72 °C for 60 sec) \times 35 cycles, 72 °C for 10 min. We visualized the PCR products on a 1% agarose gel and cleaned them with Exo-Sap-It (USB, Cleveland, OH). For the sequencing reactions we used BigDye 3.1 chemistry (ABI) and the same primers as for PCR amplification. Sequences were read with an ABI 3130 automated sequencer. Our primers coamplified and cosequenced an additional pseudogene of ~100 bp, but we were able to read the entire 271 bp of the target fragment in both the forward and reverse sequences. We assembled the sequence contig (set of overlapping DNA segments derived from a single genetic source) and inspected chromatograms manually with Sequencher 4.7 (GeneCodes, Ann Arbor, MI). We used the software package MUSCLE (Edgar 2004) for alignment with Genbank sequence no. AF205605.1 and each of the 46 additional haplotypes reported by Avise et al. (2000). We used the program MEGA (Tamura et al. 2007) to calculate uncorrected pairwise distances and for distance-based phylogenetic analysis. We used the program Phylml (Guindon and Gascuel 2003) for phylogenetic analysis by maximum likelihood, using the default parameters (HKY85 model with gamma-distributed rate variation among sites) and simultaneous estimation of the model's parameters. We ran 500 bootstrap replicates of the maximum-likelihood analysis to assess support of the branch nodes.

We obtained the paths and times of tropical storms in both the eastern Pacific and Caribbean basin from the National Oceanic and Atmospheric Administration's Hurricane Center on 7 March 2011 (www.nhc.noaa.gov/2010epac.shtml; www.nhc.noaa.gov/2010atlan.shtml). We considered all tropical storms or hurricanes that moved inland from the Pacific or Atlantic toward New Mexico during the month before the bird was discovered. We also checked local weather archives for Carlsbad, New Mexico, and archived regional weather reports for Texas and New Mexico in the week before the discovery of the tern.

RESULTS

The New Mexico specimen of the Sooty Tern has nearly white underparts and nearly black upperparts (Figure 1). Its white forehead extends into a supercilium that does not extend behind the eye (Figure 1). Viewed ventrally, its primaries are uniformly gray on the inner web, slightly lighter on the outer web, lacking the extensive white in the vanes (Figure 1). This combination of characters confirms the identity of the specimen as a Sooty Tern. The specimen lacks the white in the primaries and the long supercilium extending past the eye of the Bridled Tern (*O. anaethetus*). The nearly black back rules out the Bridled and Gray-backed (*O. lunatus*) Terns, and this speci-

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men is too pale ventrally and too dark dorsally to be an Aleutian Tern (*O. aleuticus*). We identified the specimen as an adult from its plumage pattern, plumage wear, molt, and absence of a bursa of Fabricius. The measurements of wing, culmen, and tarsus were unable to eliminate any of the potential source populations (Table 1).

The molecular sequence generated from MSB 30000 was 271 bp long from the 3' end of the forward primer to the 5' end of the reverse primer. The sequence has been deposited in Genbank (accession no. HQ713543). The base positions correspond to positions 42 to 312 of the sequence of Avise et al. (2000). Pairwise comparison of MSB 30000 to the 47 haplotypes of Avise et al. (2000) revealed mean genetic distances of 0.020–0.021 (range: 0.011–0.033) from samples from Hawaii (Johnston Atoll) and the Indian Ocean (Table 2). Mean genetic distances of the New Mexico specimen to samples from Puerto Rico and Ascension Island were much lower, 0.009 (range 0.000–0.018) and 0.007 (range 0.000–0.015), respectively. The haplotype of MSB 30000 was identical to Ascension Island haplotypes AS8 and AS9 and Puerto Rico haplotype PR3 (Fig. 2) of Avise et al. (2000). The phylogenetic result shows a dichotomy corresponding to the Puerto Rico-to-Ascension Island clade and Hawaii-to-Indian Ocean clade, with MSB 30000 falling clearly in the former. Bootstrap support is low, but this is expected given the small number of informative characters in the data set. Avise et al. (2000) considered the character states at base-pair positions 131, 198, and 200 of their alignment to be diagnostic for the Caribbean and Atlantic populations of the Sooty Tern. At each of the three positions, the bases in the New Mexico bird match the state expected for Caribbean and Atlantic populations.

Only one storm, Hurricane Alex from the Caribbean Basin, passed at a time and with a track that might have carried this Sooty Tern off course. Hurricane Alex passed over the base of the Yucatán Peninsula on 27 June before making its way northwest over the southern Gulf of Mexico, then turning southeast to make landfall in central Tamaulipas and dissipating over the western edge of San Luis Potosí on 2 July 2010. Carlsbad, New Mexico, received ~100 mm rain in the week leading up to the find, as predicted by the track of Alex's remnants over northern Mexico into the southwestern United States (Sosnowski 2010).

Table 2 Estimates of Evolutionary Divergence in 271 Base Pairs of Mitochondrial DNA between Sooty Terns from Various Locations^a

	(1)	(2)	(3)	(4)	(5)	(6)
(1) Johnston Atoll, Hawaii		[0.007]	[0.007]	[0.004]	[0.004]	[0.007]
(2) Puerto Rico	0.028		[0.004]	[0.007]	[0.007]	[0.003]
(3) Ascension Island, Atlantic Ocean	0.024	0.016		[0.006]	[0.006]	[0.002]
(4) Chagos Archipelago, Indian Ocean	0.016	0.028	0.023		[0.004]	[0.007]
(5) Seychelles, Indian Ocean	0.018	0.027	0.024	0.019		[0.007]
(6) New Mexico	0.021	0.009	0.007	0.02	0.021	

^aBelow diagonal, based on the number of base differences per site, averaged over all pairs of sequences in MEGA 5 (Tamura et al. 2007). Above diagonal, standard error estimate(s). The analysis involved 47 nucleotide haplotypes published by Avise et al. (2000).

DISCUSSION

The combination of the plumage characters of our specimen of *Onychoprion* confirms its identity as a Sooty Tern. Furthermore, at the mitochondrial cytochrome oxidase I locus, Sooty Tern specimens from the Pacific Ocean differ by 5.8% from Bridled Tern specimens from Florida. Therefore it is unlikely that any Bridled Tern would contain a haplotype identical to that of a Sooty Tern at the 271-bp fragment of the control region.

In measurements, MSB 30000 is decidedly small in comparison to published measurements of the Sooty Tern (Table 1), but definitive identification of the geographic origin is not possible by morphology alone because of the plumage wear of the specimen and the broad overlap and high variability of available measurements. A thorough morphological study of the subspecies that takes into account sex and plumage wear is clearly needed.

The small size of our specimen might suggest a hybrid origin, perhaps the product of a Sooty Tern mother with a Bridled Tern father. Indeed, the wing, tarsus, and culmen measurements of MSB 30000 also fall within published measurements of the Bridled Tern (Haney et al. 1999; Table 1). There are, however, no reports of a Bridled \times Sooty Tern from the literature (McCarthy 2006). An F1 hybrid should be intermediate in most phenotypic characters (Clark and Witt 2006), whereas the plumage of MSB 30000 is consistent with a pure Sooty Tern. Therefore, we consider hybridization to be a remote possibility.

The control-region sequence of MSB 30000, although short in length, unambiguously indicates an affinity with Sooty Tern haplotypes from Puerto Rico and Ascension Island to the exclusion of those from Johnston Atoll and the Indian Ocean. This result firmly indicates that this bird did not originate in either the central Pacific or Indian oceans, and it suggests that it may have come from the Atlantic side of North America via the Caribbean Sea and Gulf of Mexico. The major uncertainty in this conclusion stems from the lack of DNA sequences from Sooty Terns of the eastern tropical Pacific Ocean, where the species breeds from the Alijos Rocks west of southern Baja California south to the Gulf of Panama (Pitman 1985, Wetmore 1965). If the Sooty Terns from the eastern tropical Pacific are so closely related to those in the Caribbean and Atlantic that they share mitochondrial DNA haplotypes, then it would be equally plausible that the New Mexico specimen may have come from the Pacific. Indeed, this vagrant from New Mexico, along with previous inland records, suggests the possibility for gene flow across the Central American land barrier. Furthermore, previous genetic analyses of Avise et al. (2000) and Peck and Congdon (2004) suggest that the Isthmus of Panama has not been a barrier to gene flow for this species, whose global genetic diversity is far more recent than the formation of the isthmus \sim 3.1 million years ago. However, we suspect that haplotype sharing and gene flow are unlikely, especially considering that a different subspecies of the Sooty Tern (*O. f. crissalis*) occurs in the eastern tropical Pacific. We further suspect that the water barrier separating populations on the Pacific coast of Middle America from those on Johnston Atoll is less significant to the Sooty Tern than is the land barrier separating Pacific from Caribbean populations.

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This occurrence marks only the second record of the Sooty Tern in the southern Rocky Mountain region, the first being from Colorado (Percival 2009). Trans-Pecos Texas has records from Jeff Davis and Brewster counties (Williams 1981). Additional inland records associated with tropical storms include one from Oklahoma (Heck and Arbour 2010) and many from throughout the eastern one third of the contiguous United States (AOU 1998). New Mexico's avifauna includes several records of Pacific seabirds, including the Ancient Murrelet (*Synthliboramphus antiquus*; Hubbard 1986; MSB 9287), Long-billed Murrelet (*Brachyramphus perdix*; Witt et al. 2010; MSB 29200), and Least Storm-Petrel (*Oceanodroma microsoma*; Zimmerman 1992; MSB 9087). There is also at least one specimen of the Pacific Brown Pelican (*Pelecanus occidentalis californicus*) from New Mexico (MSB 6815).

Sequences of mitochondrial DNA from multiple individuals of *O. f. crissalis* would be required to rule out an eastern Pacific origin of the New Mexico Sooty Tern definitively. However, weather patterns prior to its discovery southeast of Carlsbad and molecular data provide strong and corroboratory evidence that this bird arrived from the Caribbean.

ACKNOWLEDGMENTS

We thank Melody Russo and Sammie Uhrig for ensuring that this specimen was promptly brought to the Museum of Southwestern Biology after salvage. We thank Kimball Garrett for measuring specimens at the Los Angeles County Museum and providing specimen data. Philip Unitt, Chris Feare, Betty Anne Schreiber, Doug Pratt, Brandon Percival, Mark Lockwood, Sartor Williams and John Hughes all provided helpful correspondence. Kimball Garrett and one anonymous reviewer provided comments that improved the manuscript.

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Accepted 30 September 2011