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Modest genetic differentiation among North American populations of *Sarcocystis neurona* may reflect expansion in its geographic range

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Abstract

Sarcocystis neurona is an important cause of neurological disease in horses (equine protozoal myeloencephalitis, EPM) and sea otters in the United States. In addition, EPM-like disease has been diagnosed in several other land and marine mammals. Opossums are its only definitive hosts. Little genetic diversity among isolates of *S. neurona* from different hosts has been reported. Here, we used 11 microsatellites to characterize *S. neurona* DNA isolated from natural infections in 22 sea otters (*Enhydra lutris*) from California and Washington and in 11 raccoons (*Procyon lotor*) and 1 striped skunk (*Mephitis mephitis*) from Wisconsin. By jointly analyzing these 34 isolates with 26 isolates previously reported, we determined that geographic barriers may limit *S. neurona* dispersal and that only a limited subset of possible parasite genotypes may have been introduced to recently established opossum populations. Moreover, our study confirms that diverse intermediate hosts share a common infection source, the opossum (*Didelphis virginiana*).

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Keywords: *Sarcocystis neurona*; Sea otter (*Enhydra lutris*); Raccoon (*Procyon lotor*); Skunk (*Mephitis mephitis*); Microsatellite; PCR

1. Introduction

Sarcocystis neurona was first recognized as an important cause of a neurologic disease in horses, equine protozoal myeloencephalitis (EPM) (Dubey et al., 2001a). More recently, its importance as a pathogen in sea otters has been established (Thomas et al., 2007). It also causes clinical sarcocystosis in cats, mink, raccoons, and other mammals. Opossums are its definitive hosts and other mammals act as intermediate or accidental hosts. Viable *S. neurona* has been isolated from sea otters, harbor seals, cats, opossums, raccoons, and horses (Dubey, 2000; Dubey et al., 1991, 2001b,c; Lindsay et al., 2000, 2001a; Mansfield et al., 2001; Miller et al., 2001a,b; Turay et al., 2002). The parasite is

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restricted to the Americas, coinciding with the geographic range of opossums (Dubey et al., 2001a).

Little genetic diversity has been described among isolates of *S. neurona* from its various hosts (Elsheikha et al., 2006; Elsheikha and Mansfield, 2007). One study using 12 highly polymorphic microsatellite markers (Asmundsson et al., 2006) found that *S. neurona* isolates from North America were derived from a single, intermixing population. By contrast, two South American parasite isolates were genetically distinct from *S. neurona* of North American origin (Asmundsson et al., 2006). Such markers can help resolve the historical and ongoing subdivision of biological populations because they are sampled from throughout the genome and because their alleles are presumed to be selectively neutral. Nonetheless, they sample only a portion of the genome and would not necessarily detect localized or recent changes to the genome. To further characterize the genetic structure of *S. neurona*, and to determine whether genetically distinct parasites are present in free-living wildlife populations, we amplified and characterized

Table 1
Description of *Sarcocystis neurona* isolates and genotypes

Host	ID	Geographic origin	Date Collected	Sn1	Sn2	Sn3	Sn4	Sn5	Sn6	Sn7	Sn8	Sn9	Sn10	Sn11
Raccoon	358	WI	01/05/2006	180	198	231	187	225	254	158	200	190	167	174
Raccoon	359	WI	01/07/2006	182	199	231	187	225	254	160	200	190	167	174
Raccoon	362	WI	01/08/2006	180	199	229	185	225	254	158	200	190	167	174
Raccoon	363	WI	01/08/2006	180	198	231	185	225	254	160	200	190	167	174
Raccoon	365	WI	01/08/2006	182	198	231	187	225	254	160	200	190	167	174
Raccoon	370	WI	01/08/2006	180	F	231	185	227	254	160	200	190	167	174
Raccoon	387	WI	01/14/2006	180	198	231	185	225	250	160	200	190	167	174
Raccoon	391	WI	01/25/2006	182	199	231	187	225	254	160	200	190	167	174
Raccoon	393	WI	01/27/2006	180	198	231	185	225	254	160	200	190	167	174
Raccoon	412	WI	01/31/2006	180	198	231	185	225	254	160	200	190	167	174
Raccoon	413	WI	02/06/2006	182	198	231	187	225	254	160	200	190	167	174
Skunk	365	WI	10/20/2005	180	199	231	187	225	250	160	200	190	167	174
Sea Otter	16227	Monterey, CA	04/1999	184	197	231	187	225	246	162	200	190	165	174
Sea Otter	19030	WA	04/2004	180	198	231	187	227	252	164	200	190	167	174
Sea Otter	16445	Santa Cruz, CA	10/1999	182	196	229	187	225	252	166	200	190	165	172
Sea Otter	15821	Santa Cruz, CA	06/1998	184	196	233	187	225	250	162	200	199	169	174
Sea Otter	16904	WA	07/2000	180	199	231	187	225	252	F	200	190	167	174
Sea Otter	19057	WA	05/2004	180	199	231	187	225	252	162	200	190	167	172
Sea Otter	18096	Monterey, CA	03/2002	F	196	233	185	225	250	158	200	190	169	174
Sea Otter	15822	Santa Cruz, CA	06/1998	184	196	233	185	227	250	158	200	199	169	174
Sea Otter	15713	WA	03/1998	184	199	231	187	225	252	162	200	190	167	172
Sea Otter	15792	Santa Cruz, CA	05/1998	184	196	233	185	225	250	164	200	199	169	174
Sea Otter	14675	Santa Cruz, CA	01/1997	184	197	229	187	227	252	158	200	190	165	174
Sea Otter	13631	San Luis Obispo, CA	05/1995	F	196	231	F	227	250	F	200	196	165	174
Sea Otter	14414	San Luis Obispo, CA	08/1996	188	199	231	187	225	250	168	200	196	165	172
Sea Otter	11429	Santa Cruz, CA	03/1993	182	196	229	187	225	252	164	200	190	165	174
Sea Otter	11450	San Luis Obispo, CA	04/1993	182	199	231	187	227	242	162	200	190	F	174
Sea Otter	12749	Santa Cruz, CA	04/1994	184	197	231	185	225	250	158	200	199	169	174
Sea Otter	12712	Santa Cruz, CA	04/1994	184	197	231	185	227	250	158	200	199	169	174
Sea Otter	10696	San Luis Obispo, CA	04/1992	F	F	233	185	227	244	F	200	190	F	174
Sea Otter	13502	WA	03/1995	182	197	229	191	225	242	162	200	190	165	174
Sea Otter	13590	San Luis Obispo, CA	04/1995	F	199	229	187	225	F	168	200	196	165	F
Sea Otter	14226	San Luis Obispo, CA	04/1996	184	197	231	185	225	250	158	200	199	169	174
Sea Otter	13479	San Luis Obispo, CA	03/1995	F	198	231	187	227	250	168	200	196	165	F

The columns of each locus (Sn1–Sn11) indicate the estimated number of base pairs amplified. *F* = amplification failure.