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Discernible but limited introgression has occurred where *Trichinella nativa* and the T6 genotype occur in sympatry

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ABSTRACT

The genetic diversity within and among parasite populations provides clues to their evolutionary history. Here, we sought to determine whether mitochondrial and microsatellite DNA variation could be used to evaluate the extent of differentiation, gene flow and historical reproductive isolation among the freeze resistant parasites *Trichinella nativa* and the *Trichinella* T6 genotype infecting wolverines (*Gulo gulo*) in Nunavut, Canada. To this end, we genotyped *Trichinella* isolates derived from the diaphragms of 39 wolverines from this locale to reference strains of *T. nativa* and the *Trichinella* T6 genotype. Results showed that among a subset of 13 isolates examined, individuals resembled *T. nativa* in their mitochondrial DNA, but resembled the *Trichinella* T6 genotype when assayed at expansion segment V and the internal transcribed spacer of the nuclear rDNA. To adjudicate among these conflicting diagnoses, we further characterized each isolate at several nuclear microsatellite loci and again compared these to data from reference strains. Statistical assignment established that the nuclear genomes of most Nunavut isolates corresponded to those of the *Trichinella* T6 genotype; however, two isolates corresponded to *T. nativa*, and one isolate exhibited equal similarity to both reference strains. Taken as a whole, the evidence suggests that these isolates derive from the *T. nativa* matrilineage, but that their nuclear genomes resemble individuals previously designated as *Trichinella* T6. Assuming distinct lineages, this argues for cross-hybridization among these genotypes. Although introgression has occurred, recognizable genetic distinctions persist. One possibility is that selection disfavors the survival of hybrid offspring in most instances. Alternatively, the recent disappearance of glacial barriers might have increased contact, and therefore introgression. Broader geographic sampling will be required to determine the extent to which hybridization occurs beyond this particular geographic focus.

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1. Introduction

Human trichinellosis is contracted by ingesting uncooked meat containing encysted larvae of *Trichinella* spp. Although diagnosis can be difficult owing the variability of its clinical presentation and their resemblance to other pathological processes, common symptoms (in the acute phase, during larval migration from the gut to muscle tissues) often include gastroenteritis, abdominal pain, myalgia, and eosinophilia, tissue edema, vasculitis, and intravascular thrombi (Gottstein et al., 2009). These parasites infect a variety of mammalian, avian, and reptilian omnivores and carnivores (Pozio et al., 2009). Although most human infections are acquired by eating pork infected with *Trichinella spiralis*, wild game can represent an important source of food borne risk, especially in communities, such as those in the Canadian Arctic, where wild game is frequently consumed (Eaton, 1979; MacLean et al., 1989, 1992; Kapel, 1997; Serhir et al., 2001).

Two distinct taxa of *Trichinella* have been identified as endemic to the Canadian Arctic: *Trichinella nativa* and the *Trichinella* T6 genotype (hereafter referred to simply as 'T6'). Phylogenetic analysis attests to an especially close relationship between these genotypes, which share similar genetic and biological characteristics such as freeze resistance (Pozio and Zarlenga, 2005). Natural hybrids between these taxa have been identified (La Rosa et al., 2003) and larvae endemic to the Arctic are freeze resistant and capable of surviving prolonged periods within the frozen tissues of carnivores (Pozio et al., 1992; Kapel et al., 1995, 1999; La Rosa et al., 2003; Malakauskas and Kapel, 2003; Davidson et al., 2008; Reichard et al., 2008; Gajadhar and Forbes, 2010). Because only molecular and biochemical methods can be used to differentiate

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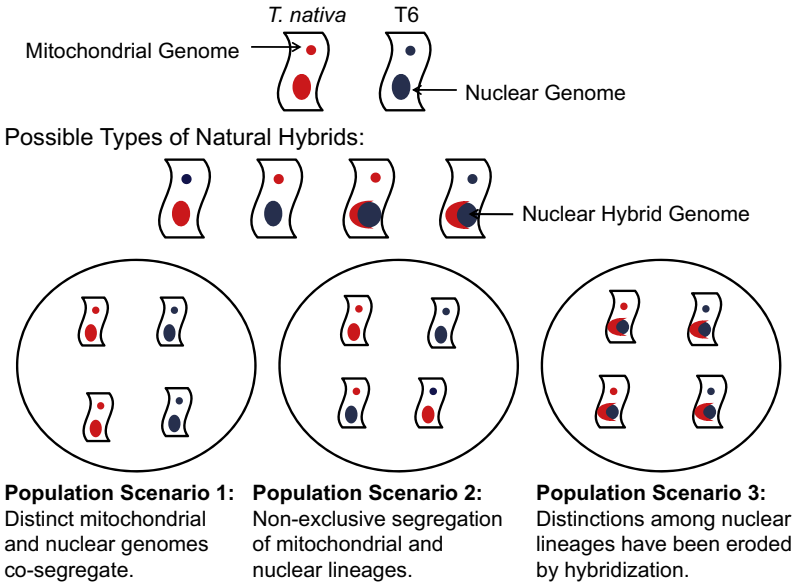

Fig. 1. A schematic of genetic variation distributed among sympatric forms of *Trichinella*. A failure of hybrid offspring to form, survive, or reproduce would lead to the persistence of distinct nuclear genomes, each demarcated by its own corresponding mtDNA haplotype. In the event of hybridization, nuclear genomes can become admixed but mitochondrial genomes are inherited as a non-recombinant haplotype, resulting either in the superimposition of two distinct mitochondrial haplotypes on the same background of nuclear variation (if hybridization is rare and back-crosses are common), or resulting in the erosion of previous distinctions between nuclear lineages.

it from *T. nativa*, the T6 genotype has not yet been recognized as a distinct species (Pozio et al., 1992; Zarlenga et al., 1999, 2001; La Rosa et al., 2003; Reichard et al., 2008). Experimental studies have shown that the F1 progeny of a cross between a female ascribed to *T. nativa* and a male ascribed to T6 are infertile (La Rosa et al., 2003). Recently, a study of 41 wolverines (*Gulo gulo*) from Canada employed a diagnostic assay based on variation in nuclear rDNA (Reichard et al., 2008) and diagnosed 33 wolverines with T6 alone, two with both *T. nativa* and T6, and one with *T. nativa* alone.

In principal, contact between genetically-differentiated, sympatric lineages such as *T. nativa* and T6 could result in any of several outcomes (see Fig. 1): sympatric species could remain entirely reproductively isolated and maintain distinct nuclear and mitochondrial genes; rare introgression and frequent back-crossing could result in the transmission of only one mitochondrial haplotype to hybrid offspring that retain the other lineage's nuclear genes, or; frequent hybridization could erode distinctions between the nuclear genomes, compromising the capacity of any single marker to differentiate between them.

Here, we sought to further examine the extent and nature of gene flow between these two parasite taxa in a locale of established sympatry. In particular, we characterized a suite of nuclear microsatellite (μsat) markers and a portion of their mitochondrial genomes to assess whether parasites from these wolverines in Nunavut, Canada indeed comprise two, reproductively independent populations differentiable by specified genetic attributes, or instead demonstrate evidence of introgression.

2. Materials and methods

2.1. Sample collection, isolation, and DNA extraction

We employed previously described methods to characterize variable genetic markers from pools of larvae digested from infected animals, and also modified these procedures to verify their performance on individual larvae. As described below, our analyses probed genetic information from 39 pools of larvae, each from a different wolverine, and 18 individual larvae (three from each of six wolverines). Among these individual larval DNAs, six were chosen for whole genome amplification (WGA).

First, muscle larvae of *Trichinella* spp. were recovered from 39 wolverines in the Nunavut, Canada between November 2006 and April 2007 (Table 1 and Fig. 3) by pepsin: HCl digestion of the diaphragm, tongue, masseter, quadriceps, and deltoids. Separate pools of larvae obtained from each animal were stored in vials containing 70% ethanol for 3 years prior to commencement of this study. DNA was extracted using the DNAeasy protocol (Qiagen Corp.) after decanting most of the ethanol from each vial and evaporating residual storage fluid by means of vacuum centrifugation for 30 min.

DNA was also extracted from three individual larvae from each of six isolate pools (KU 5, 7, 10, 13, 20, and 33; see Table 1), using the DNA IQ System Tissue & Hair Extraction Kit (Promega Corp.) according to manufacturer recommendations. Of these three larval extracts, the one producing the strongest amplification product in an initial PCR screen using the TP19 μsat locus (Rosenthal et al., 2008) was selected to undergo WGA using the GenomePlex Single Cell Whole Genome Kit (Sigma–Aldrich) in order to determine whether this procedure would increase the number of loci from which microsatellites could be called from larvae that had undergone prolonged storage in ethanol. For WGA, 2 μl of the primary extract was diluted 5-fold, 9 μl of which was subsequently used as template. Of the 75 μl total, 15 μl of this WGA reaction were purified using Qiaquick PCR purification kit (Qiagen Corp.). The final elution was performed with 50 μl of the kit's elution buffer.

2.2. Genetic analyses

2.2.1. Multiplex assay for variation in nuclear ITS-2

PCR amplification of the expansion segment V (ESV) and the internal transcribed spacer 1 (ITS-1) of the nuclear large subunit rDNA (LSU rDNA) was performed on 13 larvae derived from wolverines in Nunavut, as well as on reference isolates of *T. nativa* and T6 (listed in Table 1) using a previously described multiplex PCR assay (Zarlenga et al., 1999). These products were separated