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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
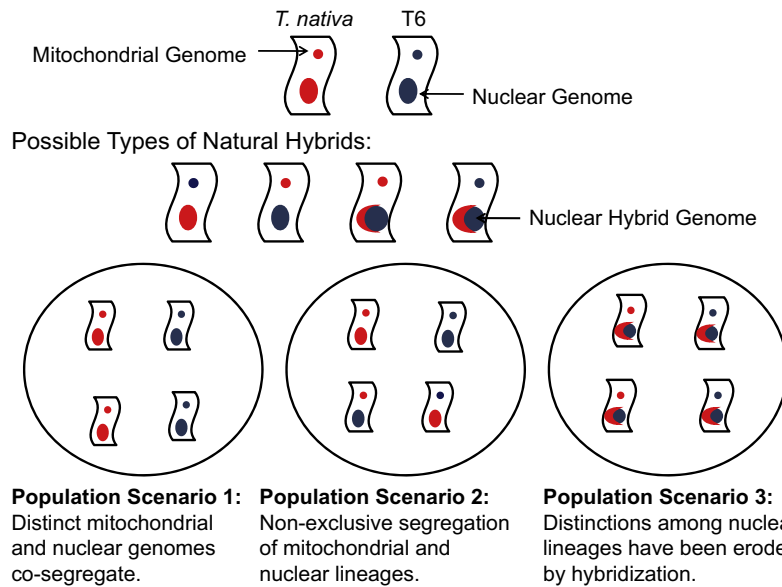
Fig. 1. A schematic of genetic variation distributed among sympatric forms of *Trichinella*. A failure of hybrid offspring to form, survive, or reproduce would lead to the persistence of distinct nuclear genomes, each demarcated by its own corresponding mtDNA haplotype. In the event of hybridization, 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

Table 1
Comparison of a subset of isolates characterized at by all three genotyping methods (multiplex, microsatellite, and mitochondrial DNA).

	Isolate	Reference (IT Code)	Country	Host	Designation based on		
					Multiplex	Microsattelite	mtDNA
Reference strains	*T. nativa* EU090	70	Russia	Wolf	*T. nativa*	*T. nativa*	*T. nativa*
	T. nativa AS092	410	Arctic	Polar bear	*T. nativa*	*T. nativa*	*T. nativa*
	T. nativa EU093	558	Finland	Raccoon dog	*T. nativa*	*T. nativa*	*T. nativa*
	T. nativa NA095	43	Canada	Polar bear	*T. nativa*	*T. nativa*	*T. nativa*
	T. nativa NA096	45	Canada	Fox	*T. nativa*	*T. nativa*	*T. nativa*
	T6	40	USA	Cougar	T6	T6	T6
	T6	g73[*]	USA	Grizzly bear	T6	T6	T6
	T6	g72[*]	USA	Fox	T6	T6	T6
	T6	g343[*]	USA	Wolf	T6	T6	T6
Pooled prior to msat genotyping	KU 07	1876	Canada	Wolverine	T6	T6	T6
	KU 08	1877	Canada	Wolverine	T6	T6	*T. nativa*
	KU 22	1891	Canada	Wolverine	T6	T6	*T. nativa*
	KU 24	1893	Canada	Wolverine	T6	T6	*T. nativa*
	KU 32	1900	Canada	Wolverine	T6	T6	*T. nativa*
	KU 35	1902	Canada	Wolverine	T6	*T. nativa*	*T. nativa*
	KU 41	1905	Canada	Wolverine	T6	T6	*T. nativa*
Genotyped individually in all assays	KU 05 L1	1874	Canada	Wolverine	T6	T6	*T. nativa*
	KU 07 L1	1876	Canada	Wolverine	T6	T6	*T. nativa*
	KU 10 L1	1879	Canada	Wolverine	T6	T6	*T. nativa*
	KU 13 L2	1882	Canada	Wolverine	T6	T6	*T. nativa*
	KU 20 L1	1889	Canada	Wolverine	T6	T6	*T. nativa*
	KU 33 L2	1898	Canada	Wolverine	T6	T6	*T. nativa*

Top panel: reference isolates of *T. nativa* and the T6 genotype. Middle panel: isolates from Nunavut, Cananda, genotyped as individual larvae using the multiplex and mitochondrial assays, but pooled prior to genotyping with microsatellites. Bottom panel: isolates from Nunavut, Canada genotyped as individual larvae using all three systems.
[*] ITRC ISS code not assigned for g72, g73, or g343.

and visualized using the QiAxcel electrophoresis system (Qiagen Corporation).

2.2.2. Microsatellite assays

To analyze the 39 extracts derived from pooled larvae using nine allelic μsat markers, we employed methods that were previously used to genotype a large sample of *Trichinella* spp., including seven isolates of *T. nativa* and one isolate of the T6 genotype (Rosenthal et al., 2008). That study, which identified a notable deficit of variability in the *T. spiralis* endemic to Europe and the Americas, completely genotyped all eight aforementioned isolates of *T. nativa* and T6. Among those isolates, all loci were variable, ranging from 2 to 8 alleles/locus (mean 4.4, se 2.2), 17 of 72 (24%) individual allele calls were heterozygous, and each of the eight isolates bore a unique multilocus genotype. To analyze these markers from extracts derived from individual larva, these methods were modified as follows: 2.5 μl of template were used in 40 cycle PCRs. To assess the optimal volume of such products to be analyzed on an ABI 3730 capillary electrophoresis instrument, various volumes of the PCR products (2 μl, 1 μl, and a 1 μl of a 50-fold dilution) were mixed with HiDi Formamide containing 0.75% GeneScan 500 LIZ molecular weight standards (Applied Biosystems) to a total volume of 10 μl.

Newly sampled isolates were compared to each other, and to those samples previously reported by Rosenthal et al. (2008). In total, 168 isolates were compared at the nine loci. Structure (v 2.3.3) was used to assign individuals to any one of K populations, using the admixture model of ancestry and using an initial Dirichlet parameter of 1.0, a uniform prior, and a maximum alpha value of 10.0. The SD for updating alpha was set at 0.025. Allele frequencies were assumed to be correlated among populations with a mean prior Fst of 0.01, a prior SD of Fst of 0.05, a constant Lambda set initially at 1.0. We estimated the probability of the data under the model and used 10 as the frequency of Metropolis update for Q; K was varied from 3 to 10. Each run commenced with a burn in period of 100,000 and sampled from a posterior distribution composed of 1 million generations. The probability of the data under

each model was evaluated 10 times, and $K = 7$ was selected as a model that captured sufficient complexity without unduly inflating variance terms, using the method described by Yang et al. (2005).

2.2.3. Mitochondrial DNA assays

Approximately 1800 bps of the mitochondrial genomes from seven isolate pools (KU 7, 8, 22, 24, 32, 35, and 41), as well as templates derived from six individual larvae (KU 5L1, 7L1, 10L1, 13L2, 20L1, and 33L2) were sequenced, corresponding to the first half of the region employed in a previous study (Rosenthal et al., 2008). Similar procedures were applied to several reference isolates of T6 and *T. nativa* (Table 1) and a reference isolate of *murrelli*. The 1800 bps was generated from three amplified DNA fragments using the following primer combinations; Trich-cob-F1 (Lavrov and Brown, 2001) and Trich-cob-R3 (973 bp), Trich-seq-F2 and Trich-seq-R4 (938 bp), or Trich-seq-F3 and Trich-seq-R5 (1027 bp) (Table 2). The same primers were used for DNA cycle sequencing. BLAST was employed to verify the resulting sequences as homologous to the target.

Matrilineal descent relationships were reconstructed from mitochondrial DNA sequences under the criterion of Minimum Evolution as implemented in MEGA v4.1 (Tamura et al., 2007; Kumar et al., 2008). Evolutionary distances were computed using the Maximum Composite Likelihood method and are presented as the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the analysis,

Table 2
Primer sequences for amplifying and sequencing mitochondrial DNA.

Primer	Sequence
Trich-cob-F1	CAATCCATTAGGTACACACTCAC
Trich-seq-F2	CACATGATTCACAATCACCT
Trich-seq-F3	GCAGTAAGAAACCCATCAGA
Trich-seq-R3	TAAGTAAGATTTCAATGGCG
Trich-seq-R4	AAATCTTAAGTACTCGTAGTTTA
Trich-seq-R5	AGGTTGTGATTATTAGTTTCTAGGG

resulting in a total of 1160 positions in the final dataset including product from individual and pooled larvae.

Microsatellite and multiplex markers as well as mitochondrial DNA sequences from all wolverine isolates were compared to known reference isolates of *T. nativa*, T6, and other congeners (Table 1, Fig. 3). To better contextualize the range of variation in microsatellite markers, the present sample was also compared to those isolates of other species of *Trichinella* previously analyzed Rosenthal et al. (2008).

3. Results

3.1. Nuclear rDNA

From each of the 13 larvae assayed from wolverines in Nunavut using the multiplex PCR assaying variation in nuclear rDNA (Zarlenga et al., 1999), two bands were amplified whose lengths corresponded to those amplified from reference isolates of the T6 genotype (Fig. 2). In previous assays of 10 other larvae digested from wolverine KU-35, some resulted in the amplification of two bands, whereas others resulted in the amplification of only one (Reichard et al., 2008).

3.2. Microsatellite assays based on pooled larvae, individual larvae, and individual larvae subjected to whole genome amplification

A scorable was achieved in 336/351 (96%) attempts from the 39 extracts derived from pooled larvae (Fig. 3). Observed heterozygosity ranged among loci from 2% to 74% (mean 30%, se 26%). True heterozygosity may have been greater, if null alleles resulted in misclassifications of heterozygotes as homozygous. The contribution of such null alleles can be estimated from the frequency of 'double nulls' (15% in this dataset); doing so, however, requires strong assumptions about the degree of inbreeding taking place (for example, null alleles would be estimated at a frequency of 39% if random mating were assumed; however, frequent inbreeding is expected for species of *Trichinella* because larval cohorts must often derive from synchronous exposure to closely related parents; La Rosa et al., in press).

Of the 18 extracts derived from individual larva, 14 were successfully amplified in an initial screen targeting μsat locus 1. Overall, the genotyping success of these individual larval extracts was poor (mean number of loci amplified = 4.28, variance 1.64), perhaps because they had been stored for a considerable period in ethanol prior to DNA extraction. From 10 of these 14 larvae, genotyping was possible at 4 or more of the 9 attempted loci. Six of these 14 templates, each derived from a different wolverine, were subsequently chosen to undergo WGA. Doing so improved the number of μsat loci amplified (mean = 7.2, variance 2.16), rendered all six capable of being genotyped at 4 or more of the 9 loci, enabled two of the six to be completely genotyped, and enabled one other to be genotyped at 8 of the 9 loci.

3.3. Population assignment and phylogenetic reconstruction based on variation in nuclear microsatellites

Qualitative assessment of the distribution of microsatellite variation (Fig. 3) identifies notable and exclusive similarity between isolates from Nunavut and reference isolates of T6 (locus 5, 6, and 8). At other loci, discrimination is not possible owing to the fact that reference isolates are variable, encompassing alleles that are also observed in Nunavut samples (1, 2, 4) or that are distinct from those most common in Nunavut samples (7, 9). At one locus (3) the same two alleles, in relatively equivalent proportions, occur in isolates from Nunavut and those of *each* reference taxon.

The data depicted in Fig. 3 were then subjected to statistical analysis to identify subdivisions among the isolates. On the basis of assaying alleles of nuclear microsatellites, nearly all isolates (larval pools) corresponded to reference isolates of T6 (depicted in dark blue, Fig. 4; identified in Table 1). This broadly agreed with a prior assessment of infections in these wolverines based on application of multiplex PCR to individual larva, wherein all infected wolverines but three were diagnosed with T6, exclusively (Reichard et al., 2008). By contrast, a small subset of isolates aligned with reference specimens of *T. nativa* that aggregated in a separate population (depicted in red, Fig. 4). In two cases (KU 02 and KU 35), wolverines in which larvae of *T. nativa* had been diagnosed on the basis multiplex analysis were assigned with high-level agreement to *T. nativa* when analyzed as pools with microsatellite alleles. However, the prior work had also identified, in these two wolverines, parasites corresponding to T6; their contribution was not evident from the analysis of microsatellite data. Pooled larvae from another wolverine (KU 40), that had previously been diagnosed with *T. nativa* alone, supported amplification of microsatellites assigned with 50% probability to *T. nativa* and 50% probability to T6. (These three cases are indicated by arrows in Fig. 4). Microsatellite genotyping of individual larvae resulted in exclusive assignment to T6 in all but two cases. In one such larva (KU 05 L1), the affinity to T6 was 80% whereas the affinity to *T. nativa* was 20%. Thus, the present approach broadened the sampling of the nuclear genome, but (when applied to pools rather than individuals), failed to discern two cases of established admixture.

A neighbor-joining tree was reconstructed from a matrix of pairwise dmu^2 distances (Goldstein et al., 1995) as implemented by Populations v.1.2.30 (Fig. 5). This analysis, which is sensitive to biases induced by missing data, was restricted to templates that were completely genotyped at all μsat loci (including 32 larval pools and two individual larva derived from wolverines in Nunavut, as well as larval pools derived from four reference isolates of T6, seven reference isolates of *T. nativa*, and 43 reference isolates from outgroup taxa). This analysis generally allied isolates from these wolverines with reference isolates from T6. For some isolates (i.e. KU 24) this affinity was pronounced (represented by an especially short branch); less affinity (depicted by longer intervening branches) was recognized for others (including KU 02 and 35, identified above as resembling *T. nativa* as judged by Structure).

3.4. Mitochondrial DNA sequencing

Mitochondrial loci were consistently amplified from all templates, including those derived from individual larva that had not undergone WGA.

A subset of these (Fig. 6) were sequenced and compared over 1170 bps of mitochondrial DNA. These derived from seven pooled isolates and six individual larva infecting wolverines in Nunavut,
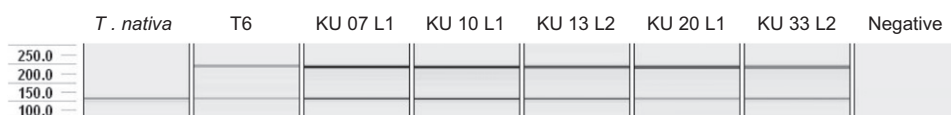

Fig. 2. Multiplex PCR of Nunavut-derived *Trichinella* DNA. Amplification products of five representative individual larvae, as well as reference isolates for *T. nativa* and T6, are shown. Each individually assayed larva (*n* = 13) corresponded to T6 when assayed with the multiplex PCR. No amplification resulted from PCR reactions lacking DNA template.
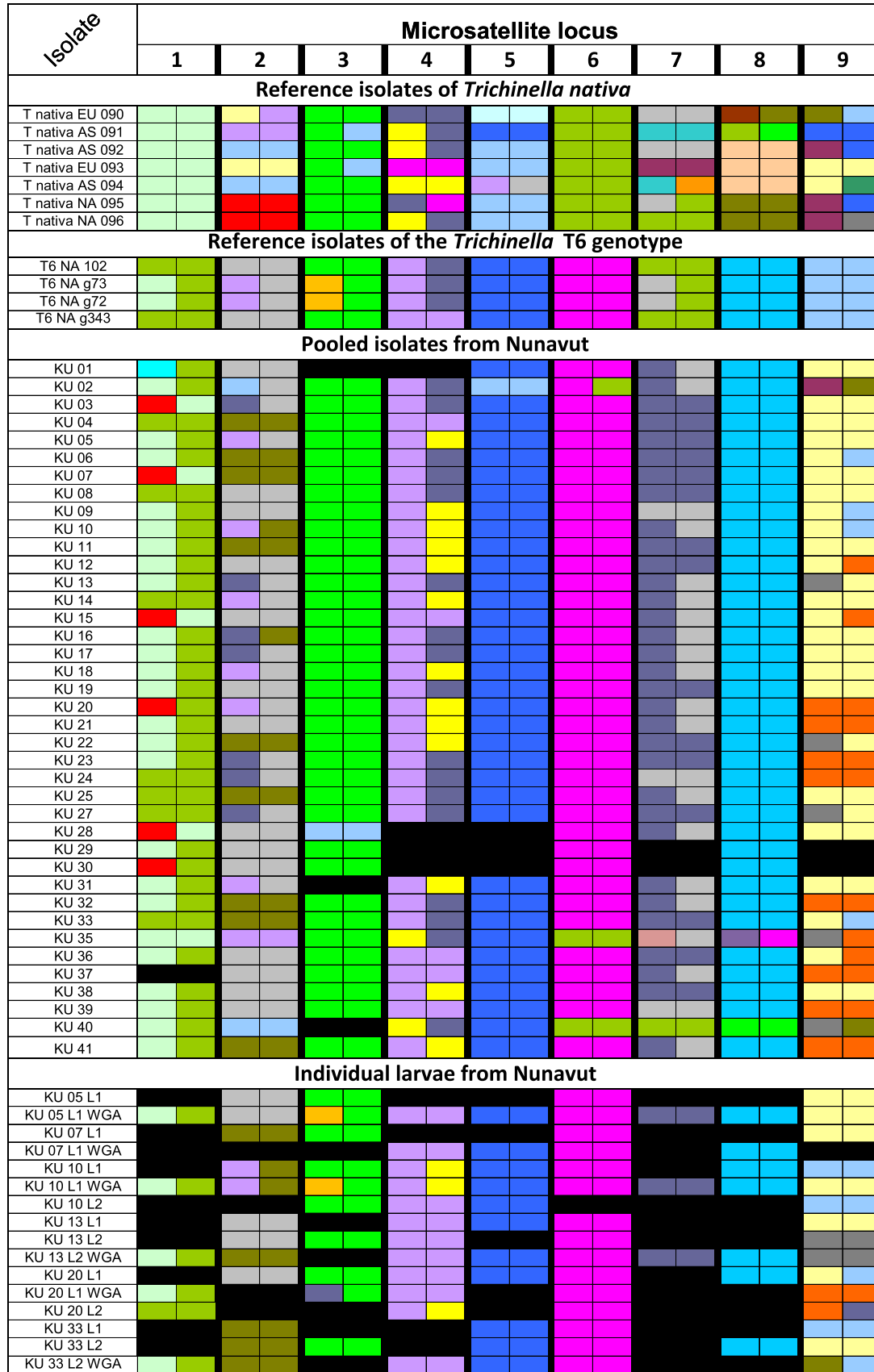
Fig. 3. Schematic representation of allele calls at each of nine μsat loci. Each size variant was arbitrarily designated with a distinct color (black = no allele call). Top panels: reference isolates of *T. nativa* and T6. Central panel: genotypes derived from pooled larvae from wolverines in Nunavut. Bottom panel: genotypes derived from individual larvae from Nunavut (including those, designated WGA that had undergone whole genome amplification prior to PCR amplification). Qualitative assessment identifies some loci (i.e. 5, 6, and 8) where isolates from Nunavut clearly bear alleles characteristic of reference isolates of T6 but not reference isolates of *T. nativa*. At other loci, such discrimination is not possible owing to the fact that reference isolates are variable, encompassing alleles that are also observed in Nunavut samples (1, 2, 4) or that are distinct from those most common in Nunavut samples (7, 9). At one locus (3) a particular allele is characteristic of Nunavut isolates *and also* those of each reference taxon.
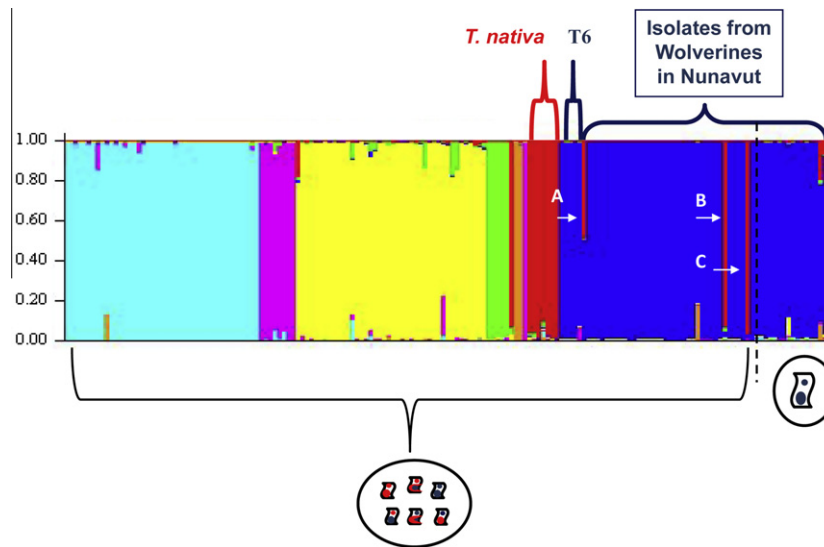
Fig. 4. Assignment of *Trichinella* isolates from Nunavut using Bayesian statistical procedures. Bayesian statistical procedures (Structure 2.3.1) unambiguously assigned most of the wolverine *Trichinella* isolates to T6 based on variation in nuclear encoded microsatellites. *Trichinella nativa* parasites are in red, T6 parasites are in blue. Arrows indicate rare isolates that were assigned with partial (A = KU 40) or strong (B = KU 02; C = KU 35) probability to *T. nativa*. The data from Rosenthal et al. (2008) were included for comparative purposes; *T. spiralis* from Europe and the Americas (turquoise); *T. spiralis* from Asia (purple); *T. britovi* from Europe (yellow); *T. murrelli* from North America (green); *Trichinella* T8 (red) and *T. nelsoni* from Africa (orange); *Trichinella pseudospiralis* from Russia (purple). Assignments were derived from larval pools (left of the dashed lines) or individual larvae (right of the dashed lines).

Canada. In addition, we sequenced 4 reference isolates from North American T6, and five isolates representing *T. nativa* derived from Arctic regions of Europe, Asia, and North America (Table 1) (GenBank Accessions JQ430659–JQ430674, JQ511986–JQ511990 and JQ513385). Doing so identified eight single nucleotide polymorphisms that segregated together, defining exactly two haplotypes. In every instance, each of the five reference isolates of *T. nativa* (Table 1, Fig. 6) bore the same haplotype; this haplotype was distinct from the haplotype shared by each of four reference isolates of T6 (Table 1, Fig. 6). From wolverines in Nunavut, all 13 sequenced templates (whether derived from pooled or individual larvae) corresponded perfectly to the haplotype ascribed to *T. nativa*. Phylogenetic reconstruction of variation in these sequences shows that a subset of Nunavut isolates and *T. nativa* share common maternal ancestry (Fig. 6).

4. Discussion

Previous reports have found wild carnivores infected with natural hybrids of *T. nativa* and T6 (Pozio et al., 2009).

The history of *Trichinella* spp. can be successfully interrogated by analyzing the extent and distribution of genetic variation. For example, the basic relationships and modes of diversification among parasites in the genus were inferred from such data (Zarlenga et al., 2006) and affinities in mitochondrial DNA and microsatellite loci substantiated the conclusion that *T. spiralis* was introduced to the Americas from Europe (Rosenthal et al., 2008). Here, we employed similar methods to address the genetic relationship between *T. nativa* and T6. In particular, we sought to determine the extent and nature of regional gene flow that may be occurring between these lineages where both types occur. Genotyping with mtDNA enabled us to trace the maternal ancestry of these isolates, whereas μsat genotyping provided a means to assess whether interbreeding has eroded historical barriers to nuclear gene flow.

For over a decade, isolates of *Trichinella* have been discriminated by interrogating variation in the ITS and ESV of nuclear rDNA (Zarlenga et al., 1999). Using this assay, a common band is

amplified from all freeze-resistant isolates. However, a second product, derived from the ITS-1, has been taken as specifically diagnostic for isolates of T6. The present study employed this method as an initial screen for identification, but expanded upon it given the insufficiency of any single locus to describe the extent and character of gene flow that may be occurring.

In each of the thirteen isolates from Nunavut tested here (Table 1), applying this standard molecular diagnostic tool resulted in the amplification of the two bands typical of T6 (Fig. 2). The multiplex PCR data generated herein coincided well with data presented elsewhere in diagnosing these worms as T6 (Reichard et al., 2008). Likewise, allelic variation in nuclear-encoded μsat loci *generally* supported the assignment of parasites derived from these wolverines to T6; however, two isolates were assigned with near certainty to *T. nativa*, and a third isolate was assigned with 50% probability to *T. nativa* and 50% probability to T6 (Fig. 4).

A phylogenetic approach similarly aligned most of these isolates with T6 (Fig. 5). The established sister relationship between *T. nativa* and T6 (Zarlenga et al., 2006) was not recovered in this neighbor joining tree, perhaps because microsatellite variation quickly saturates over prolonged evolutionary intervals. However, all reference isolates of *T. nativa* and T6 did cluster into two discrete groups. In spite of the observation that nuclear markers generally allied parasites in our sample with reference isolates of the T6, the matrilineal descent of all 13 sequenced isolates corresponded perfectly with that of *T. nativa*. This was true when the source of the sequenced PCR product was either pooled or individual larvae.

Taken together, these data affirm the persistence of genetically-differentiated nuclear genomes and show that at least a subset of parasites in this region resembling T6 in their nuclear genomes nonetheless carry mtDNA inherited from a *T. nativa* maternal ancestor (akin to Scenario 2 depicted in Fig. 1). Thus, we can abandon as inapplicable the first scenario depicted in Fig. 1, wherein two sympatric lineages have maintained absolute genetic isolation in the vicinity of Nunavut. Clearly, hybridization has resulted in the transmission of one matrilineal haplotype to two distinct genomic backgrounds, akin to the second population scenario depicted in Fig. 1. This introgression of the *T. nativa* mtDNA haplotype is
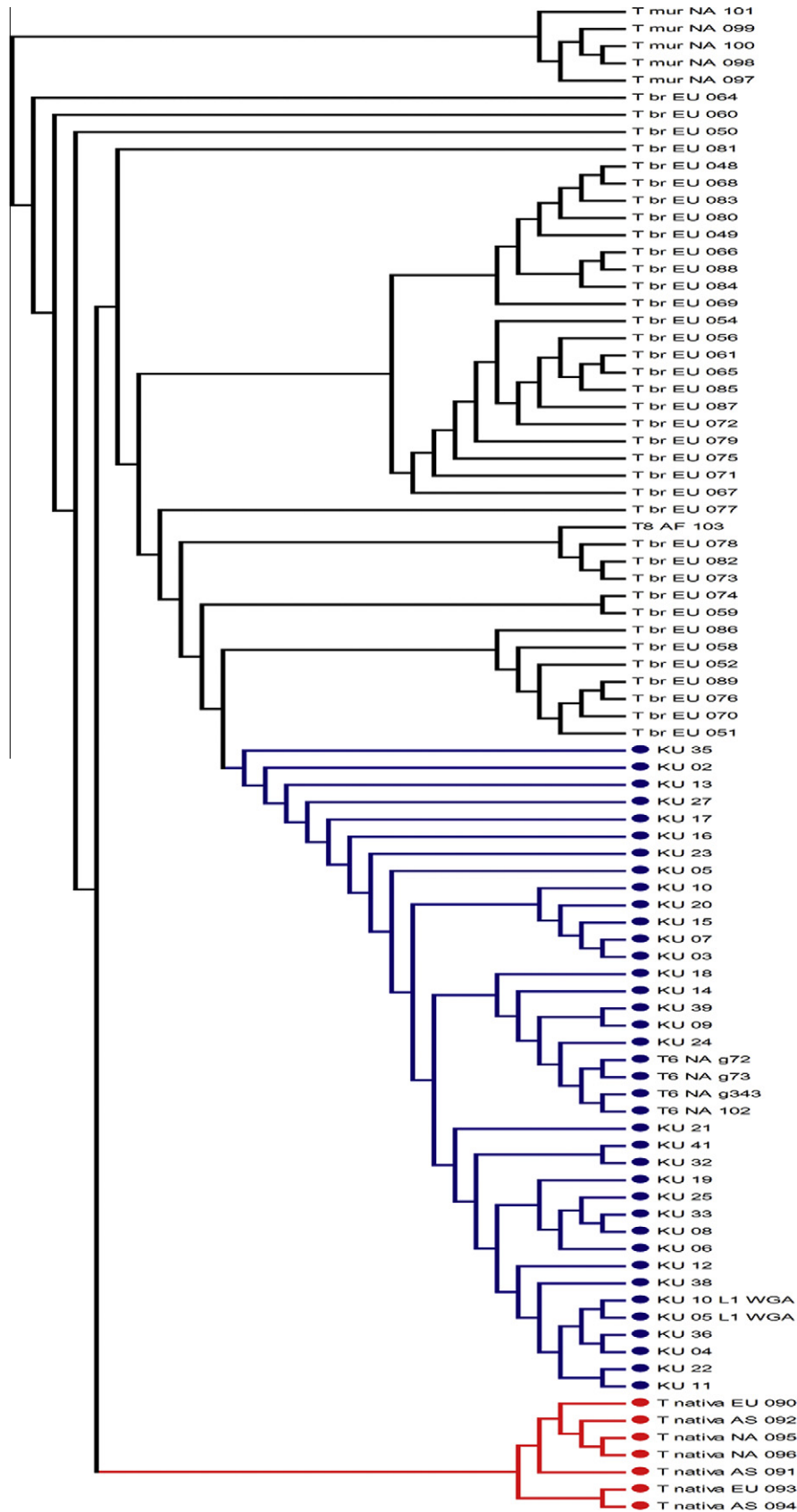
Fig. 5. Microsatellite-based phylogenetic reconstruction of *Trichinella* isolates from Nunavut. A neighbor joining tree was constructed from dmu² distances derived from μsat alleles (Goldstein et al., 1995). Blue = reference and Nunavut isolates of the *Trichinella* T6 genotype; red = reference isolates of *T. nativa*; black = reference isolates of *T. britovi* and *T. murrelli* (Rosenthal et al., 2008). OTUs represent larval pools, except as specified for two individual larvae KU 10 L1 and KU 05 L1, both of which underwent whole genome amplification prior to microsatellite genotyping.
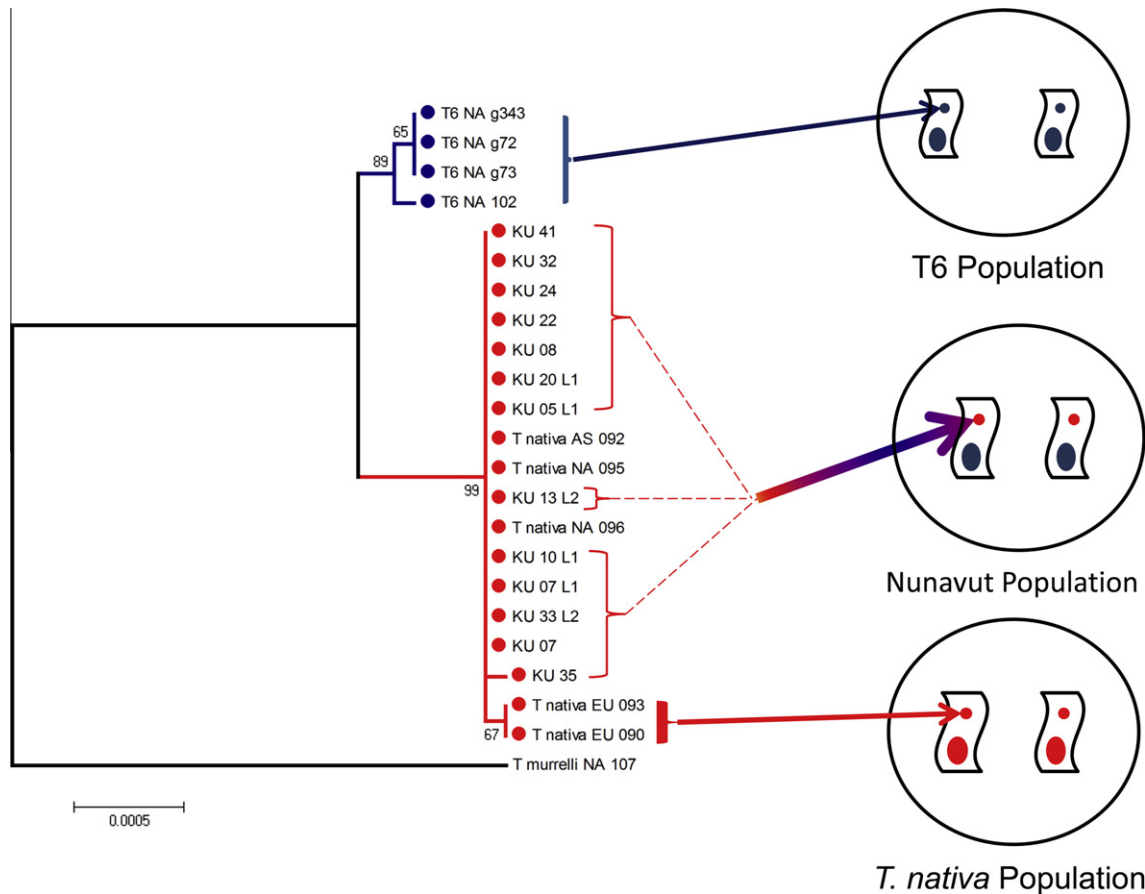
Fig. 6. Mitochondrial DNA-based phylogenetic reconstruction of *Trichinella* isolates from Nunavut. Based upon variations in mtDNA sequences and tree building, all Nunavut isolates in this study derive from the *T. nativa* matrilineage (red) rather than the *Trichinella* T6 matrilineage (blue). However, genomic DNA analyses unequivocally assign this subpopulation of isolates to the *Trichinella* T6 genotype (see large arrow).

depicted in Fig. 6. Interestingly, although these data substantiate the survival of hybrids birthed from female *T. nativa*, the offspring from exactly such experimental crosses were found to be infertile in a previous study (La Rosa et al., 2003). Perhaps the natural situation arose when F1 hybrids back-crossed with parental types. Alternatively, the genetic differentiation between *T. nativa* and T6 may not, in nature, be as complete as in the several reference isolates examined to date.

Avise (2001) reviewed many cases of introgressive hybridization spanning a broad range of plant and animal species, reflecting interesting differences in the temporal and geographic scales. Genetic admixture may or may not be confined to very narrow borders of overlap between well-differentiated taxa; introgression may be reciprocal or highly biased (in the contribution of each lineage), and may derive either from occasional transient or ongoing processes. The extent of observed mosaicism in nuclear genomes provides a means to infer the consequences of such interbreeding; 'capture' of distinct organellar genomes (such as the mtDNA of *T. nativa*, in this instance) is a frequent outcome of introgression.

More intensive sampling in Nunavut might well uncover individuals whose nuclear genomes bear attributes of both lineages (schematically represented in scenario 3 of Fig. 1). We cannot conclude from the present sample whether hybridization is rare, whether poor survival or reproduction characterizes most progeny of such hybridizations, or whether hybridization has only recently commenced. Each might explain the persistence of distinctions between the nuclear genomes of *T. nativa* and T6. We may be witnessing introgression among populations once separated by glacial barriers; given the likelihood that these lineages diverged

only recently (Zarlenga et al., 2006). It is also conceivable that shared polymorphisms are the consequence of incomplete sorting of shared ancestral variation. Resolving these alternatives would provide a rational basis for determining whether T6 warrants recognition as a distinct species, or simply as a genetic variant of *T. nativa*. Future analysis of gene flow between these two genotypes could be explored by richer sampling of additional populations of wild animals. Methods such as those used here, if applied on a broader geographic scale, could more definitively establish the origins and significance of the apparent distinction between *T. nativa* and T6.

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