Zoological Journal of the Linnean Society (2001), *131:* 443–457. With 5 figures
doi:10.1006/zjls.2000.0251, available online at http://www.idealibrary.com on IDEAL®

Genetic and morphometric variation in the Holarctic helminth parasite *Andrya arctica* (Cestoda, Anoplocephalidae) in relation to the divergence of its lemming hosts (*Dicrostonyx* spp.)

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Received February 2000; accepted for publication March 2000

Andrya arctica is a cestode parasite of the family Anoplocephalidae (Cyclophyllidea), parasitizing lemmings of the genus *Dicrostonyx* throughout the Holarctic region. The population structure of this intestinal parasite was studied from eight different regions, six of which represented different genetic entities of lemming hosts. Molecular sequence tagged site markers and minisatellite fingerprints as well as morphology and morphometrics were used to reveal the population structure of *A. arctica* in the Holarctic region. The results suggest that the evolutionary history of this cestode species has included different processes acting on different geographical regions. On the Siberian mainland (host *D. torquatus*), the division of the parasites into different genetic entities agreed perfectly with the chromosomal races of the lemming hosts that points towards a shared evolutionary history between the host and the parasite ('cospeciation'). The main phylogenetic split of *Dicrostonyx* between Eurasia and North America was not, however, observed in *A. arctica*. This suggests that in the Nearctic (host *D. groenlandicus*) the parasite has remained relatively unmodified because of the large cohesive populations ('coadaptation'). The uniqueness of the Greenland population, and possibly also that of the Wrangel Island, can be explained by peripheral isolation, refugial effects or founder effects.

ADDITIONAL KEYWORDS: Arctic–host parasite relationships – population history – DGGE – morphology – Pleistocene glaciations.

INTRODUCTION

Fluctuations of glacial and interglacial periods during the Pleistocene have promoted speciation and intraspecific divergence in the northern fauna. Isolation due to glacial barriers has led to genetic differentiation and subsequent postglacial distribution shifts have created contact zones that still persist (Hewitt, 1999).

Collared lemmings, *Dicrostonyx* Gloger, are a Holarctic genus of arvicoline rodents inhabiting the Arctic tundra; they are absent only from Fennoscandia (Jarrell & Fredga, 1993). Collared lemmings are morphologically very similar throughout the Holarctic region. Their taxonomy has not been resolved until quite recently, when chromosome surveys (Gileva, 1983; Jarrell & Fredga, 1993; Fredga *et al.*, 1995a,b) and phylogeographic patterns of mtDNA genealogies (Fedorov, Fredga & Jarrell, 1999a) revealed the divergence of this genus in the Eurasian Arctic. According to Fedorov *et al.* (1999a), the main phylogeographic split in *Dicrostonyx* is between the Palearctic and the Nearctic. Collared lemmings inhabiting the Palearctic mainland are divided into four chromosomal races and five phylogeographic clades; the two groupings are largely congruent. Wrangel Island is considered to be colonized by lemmings of Nearctic origin (Fedorov *et al.*, 1999a). All Siberian populations (Fig. 1) are now assigned to *D. torquatus* (Pallas) and those on the Wrangel Island and western Nearctic to *D. groenlandicus* (Traill) (Jarrell & Fredga, 1993). The collared lemmings of the Nearctic (Fig. 1) are currently considered as three species, *D.*

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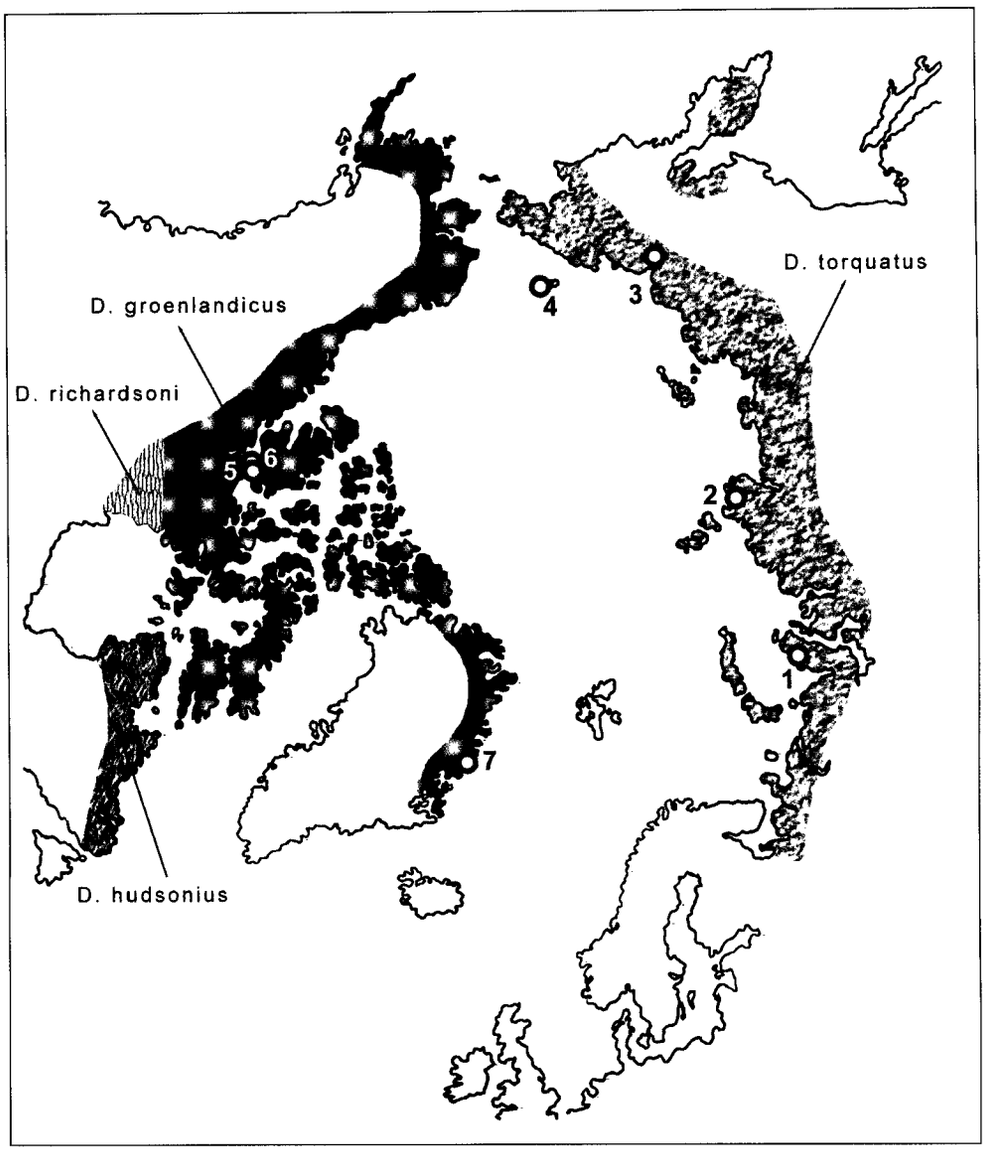

Figure 1. The distribution range of collared lemmings *Dicrostonyx* in the Holarctic. Sampling locations for this study: 1. Northern Yamal Peninsula. 2. Northern Taimyr Peninsula. 3. Kolyma River Delta. 4. Wrangel Island. 5. Byron Bay on Victoria Island. 6. Three sampling locations in the Kent Peninsula region (Walker Bay, Hope Bay and Breakwater). 7. Eastern Greenland.

groenlandicus, *D. richardsoni* Merriam and *D. hudsonius* (Pallas) (Jarrell & Fredga, 1993).

Although the genus *Dicrostonyx* is widely distributed and many aspects of its ecology and evolution have been studied (Stenseth & Ims, 1993; Fedorov *et al.*, 1999a) the existing knowledge of anoplocephalid cestodes and other helminth parasites of collared lemmings is scanty. Rausch (1952) described the species *Andrya arctica* from Alaska and Arctic Canada (host *D. groenlandicus*), and Schad (1954) reported another anoplocephalid species from Quebec and Labrador (host *D. hudsonius*). The only extensive study from the Palearctic is that of Juškov (1995), who identified two anoplocephalid cestodes of uncertain identity from northwestern Russia (host *D. torquatus*) (c.f. Haukisalmi & Henttonen, 2000).

Our ongoing studies have shown that collared lemmings have at least four species of anoplocephalid cestodes, two of which (*Andrya arctica* and *Paranoplocephala serrata* Haukisalmi & Henttonen) have a Holarctic distribution (Haukisalmi & Henttonen, 2000), and the other two (*Paranoplocephala* spp.) are restricted to the Nearctic (Haukisalmi, Wickström, Hantula & Henttonen, unpublished). All these species appear to be specific to collared lemmings, because none has been found in sympatric true lemmings

(*Lemmus* spp.) or voles of the genus *Microtus* Schrank from either the Palearctic or the Nearctic. The most common helminth species of collared lemmings is *Andrya arctica* (see Haukisalmi & Henttonen, 2000).

In concordance with refugial hypotheses based on cyclical glacials and interglacials (Hewitt, 1996; Rausch, 1994) and a model for cospeciation, we would predict the cestodes of collared lemmings to show divergence that coincides with the different genetic entities of the hosts. The aim of this study was to find out whether different genetic and morphological entities occur in *Andrya arctica* in the Holarctic region, and whether the groupings, if observed, follow the chromosomal races and phylogenetic groupings of the hosts as revealed for the Eurasian Arctic by Fedorov *et al.* (1999a) and Fedorov & Goropashanya (1999). We applied sequence tagged sites (STS) and a minisatellite fingerprints to examine genetic variation in *A. arctica*. The genetic patterns were compared with intraspecific differences in morphology and morphometrics of *A. arctica*.

MATERIAL AND METHODS

LEMMINGS

The Palearctic material of collared lemmings (Fig. 1) (*Dicrostonyx torquatus*) from the Arctic coast of Siberia and Wrangel Island ($n=81$) was collected by V. Fedorov and K. Fredga during the 'Swedish–Russian Tundra Ecology Expedition' during the summer of 1994 (Fredga, Fedorov & Jarrell, 1999). Collared lemmings were obtained from four localities, ranging from Yamal Peninsula in the west to the Wrangel Island in the east (Fig. 1). The main Nearctic material of collared lemmings (*Dicrostonyx groenlandicus*) originates from southern Victoria Island and Kent Peninsula region in Nunavut (formerly Northwest Territories), Central Arctic Canada ($n=62$) (Fig. 1). Lemmings from Arctic Canada were trapped by C. J. Krebs and A. Kenney during the summer of 1996. Lemming intestines from Siberia and Arctic Canada were frozen after trapping for later examination of helminths.

A small number ($n=3$) of intestines of collared lemmings (*D. groenlandicus*) was also obtained from Greenland; these lemmings were collected by V. Fedorov, H. P. Gelter and G. H. Jarrell on 23 July 1995, preserved entire in 70% alcohol and deposited at the University of Alaska Museum Mammal Collection (UAM NO 33909). Later, the intestines were removed from the bodies and examined for helminths.

CESTODES

Andrya arctica was found from all localities studied, its prevalence varying from 17% (Taimyr Peninsula, $n=30$) to 61% (Yamal, $n=18$). Of 39 adult worms, DNA was successfully extracted from 30 specimens (Table 1) originating from (Fig. 1) western Yamal Peninsula (73 °N, 70 °E), Taimyr Peninsula (77 °N, 105 °E), western Kolyma River Delta (69 °N, 162 °E), Wrangel Island (72 °N, 180 °E), Greenland (Ostgronland, Hurry Fjord, Hareelv, 70°42′N, 22°40′W) and four localities in Nunavut, Canada (Byron Bay 68°45′N, 109°04′W, Hope Bay 68°06′N, 106°43′W, Breakwater Island 67°55′N, 108°30′W and Walker Bay 67°N, 106°W). Two of the specimens from Yamal originated from the same lemming host, as did two from Kolyma, Greenland, Walker Bay, Hope Bay and Byron Bay. The pairs from Yamal, Kolyma, Greenland and Walker Bay had identical genetic markers, while the pairs from Hope Bay and Byron Bay possessed different markers.

After dissection, the cestodes recovered from frozen intestines were relaxed in tap water for 1–2 hours and fixed in 70% alcohol (most specimens) or 10% formalin. The specimens used in morphological analysis were stained with Mayer's haemalum or Semichon's acetic carmine, cleared in eugenol and mounted in Canada balsam. The tissue samples for genetic analysis were obtained from specimens preserved in 70% alcohol or from specimens frozen in extraction buffer (50 mM Tris/HCl, pH 7.2; 50 mM EDTA; 3% SDS; 1% beta-mercaptoethanol) prior to DNA extraction.

The material for morphometric analysis included 28 specimens of *A. arctica* from three locations: Yamal Peninsula ($n=8$), western Kolyma Delta ($n=8$) and Nunavut, Canada ($n=12$). In morphological analyses, we used specimens with gravid or pregravid segments only (pregravid segments are those immediately anterior to fully gravid segments). The specimens from other locations (Taimyr, eastern Kolyma, Wrangel Island and Greenland) were too few for proper morphometric analysis. However, we describe the main morphological features of specimens from Wrangel Island ($n=5$), because they deviated partly from the other sampling locations studied. The following representative specimens of *A. arctica* have been deposited in the US National Parasite Collection (Beltsville, Maryland): USNPC 88811 (Yamal), USNPC 88812 (Western Kolyma), USNPC 88813 (Wrangel Island) and USNPC 88814 (Victoria Island).

To determine the geographical and host distribution of *Andrya arctica*, we examined preliminarily the holotype (USNPC 37356) from Point Barrow, North Alaska (host *D. groenlandicus*) and several specimens from the personal collection of Robert L. Rausch (hosts *D. groenlandicus* and *D. richardsoni*). The detailed taxonomy of these specimens will be presented in a later publication (Haukisalmi, Wickström, Hantula & Henttonen, submitted).

MORPHOMETRIC ANALYSES

From each cestode specimen, we recorded the body length and maximum body width (pregravid segments)

Table 1. Distribution of alleles (markers STS-A and STS-B) and M13 markers (900 to 185) among *Andrya arctica* specimens. Homozygotes for STS-A or STS-B are depicted with only one number and alleles with the same migration rate were scored as equal. Groups with abbreviations starting with P and N are from the Palearctic and Nearctic, respectively. The grouping of the Palearctic individuals (P1–P3) is made according to the chromosomal races of the host

		STS		M13 markers (900–185)																			
Sampling location and country	Group	A	B	900	860	850	650	600	550	530	470	450	400	380	340	300	285	280	270	260	250	190	185
Yamal Peninsula, western central Siberia, Russia	P1	2	1/2	1*	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Yamal Peninsula, western central Siberia, Russia	P1	5	1/2	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Yamal Peninsula, western central Siberia, Russia	P1	2/5	1/2	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Yamal Peninsula, western central Siberia, Russia	P1	2/5	1/2	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0
Taimyr Peninsula, central Siberia, Russia	P1	2/5	1	0	1	0	1	1	1	0	0	0	0	0	1	0	1	0	0	1	1	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Kolyma River Delta, central eastern Siberia, Russia	P2	5	3	0	0	1	1	1	1	0	1	0	0	1	1	0	0	1	0	1	0	1	0
Wrangel Island, eastern Siberia, Russia	P3	5	5	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
Wrangel Island, eastern Siberia, Russia	P3	5	5	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
Wrangel Island, eastern Siberia, Russia	P3	6	5	1	0	1	0	1	1	0	1	1	0	0	1	0	1	0	0	1	0	1	0
Ostgronland, Hurry Fjord, Hareelv, Greenland	N1	3/5	3	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	1	0
Ostgronland, Hurry Fjord, Hareelv, Greenland	N1	3/5	3	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	1	0	0	0	1
Walker Bay, Kent Peninsula, Nunavut, Canada	N2	4	3	0	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	1	0	1	0
Walker Bay, Kent Peninsula, Nunavut, Canada	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	2	1/2	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	3	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Hope Bay, Nunavut, Canadian mainland	N2	1/2	1/2	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Breakwater Island, Nunavut, Canada	N2	1/2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3/4	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	5	3	0	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	1/2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	1	0	1	0
Byron Bay, Victoria Island, Nunavut, Canada	N3	2	3	1	0	0	1	1	1	1	0	0	0	1	1	0	0	1	0	1	0	1	0

* 1 and 0 indicate the presence and absence of the marker, respectively.

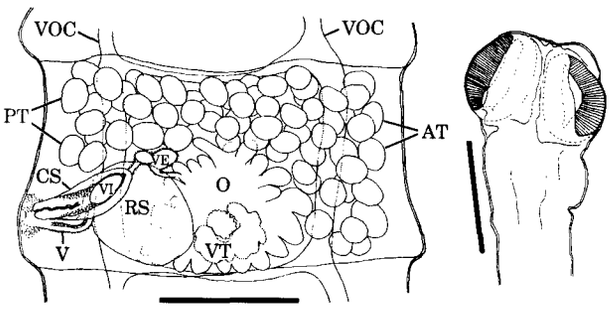

Figure 2. Mature segment and scolex of *Andrya arctica* from Victoria Island, Nunavut (host *Dicrostonyx groenlandicus*). Abbreviations: VOC, ventral osmoregulatory canal; PT, poral testes; AT, antiporal testes; CS, cirrus sac; VI, vesicula seminalis interna; VE, vesicula seminalis externa; V, vagina; RS, seminal receptacle; O, ovary; VT, vitellarium. Scale bars: 0.30 mm (segment) and 0.20 mm (scolex).

and the diameter of the scolex and suckers (if present). The width of segment, number of testes and the length and width of various reproductive organs were measured from three mature segments (Fig. 2), and the maximum length of cirrus sac and seminal receptacle were recorded from postmature segments (postmature segments are those immediately posterior to fully mature segments). The first mature segment was defined as the one in which the internal seminal vesicle was first seen clearly differentiated; the last mature segment was the one in which the vitellarium was last seen compact (no visible disintegration).

Egg size is based on the measurement of the maximum diameter of five eggs from fully gravid segments. When multiple measurements were made from a single strobila, we used the median of these values in statistical analyses. We also determined the pattern of the alternation of genital openings by counting the number of sets with unilateral segments, i.e. those having the genital pore on the same side of the segment.

We used both univariate and multivariate statistical methods to study the morphometric differences among the specimens of *A. arctica* from three sampling locations. As a first step, we performed linear regression analysis between the width of mature segments and each of the other variables. If necessary, logarithmic transformation was performed on the variables to obtain a better fit to the normal distribution. Several tests indicated a significant positive association between the two variables (Table 2). Therefore, we calculated standardized residuals from the significant linear regressions between the body width and other variables, which were then used in subsequent statistical analyses as size-adjusted morphometric measures.

The following step was to calculate rank correlation (Spearman) between various variables using either the absolute values or those adjusted for the effect of body width (residuals). Most of the variables showed significant positive correlation at least with one of the other variables, usually with several of them. We therefore performed a principal component analysis (PCA), based on a correlation matrix, for 15 variables, including the length and width of various reproductive organs and eggs (Table 3). The PCA creates new, uncorrelated variables from a set of intercorrelated variables; the new variables (principal components) are interpreted and used in subsequent statistical comparisons. The other variables were not included in the PCA either because of too many missing values (scolex, suckers and body length) or because of a lack of significant correlation with other variables (maximum length of cirrus sac and seminal receptacle). The meristic (countable) variables (genital pore alternation and number of testes) were also excluded from the PCA. The few missing values were replaced with the mean from the particular location. Variables excluded from the PCA were subjected to univariate comparisons (Kruskall-Wallis non-parametric ANOVA) using either the original values or residuals (Table 2).

DNA EXTRACTION

DNA was extracted from 0.5–2 mm^3 tissue samples as described previously (Vainio, Korhonen & Hantula, 1998). The protocol included cell disruption (using quartz sand), four phenol-chloroform (1:1) extractions, one chloroform:isoamyl alcohol (24:1) extraction, precipitation with polyethylene glycol (PEG) and drying. The DNA was resuspended in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA.

PCR AMPLIFICATION USING RANDOM AMPLIFIED MICROSATELLITE PRIMERS (RAMS)

The PCR reactions were carried out in reaction conditions recommended by the manufacturer of the Dynazyme II DNA-polymerase (Finnzymes Ltd., Finland), except that the concentration of the RAMS (Hantula, Dusabenyagasani & Hamelin, 1996) primers was 2 μM. In PCR amplifications the samples were denatured by 10 min incubation at 95°C after which 37 cycles of amplification were carried out (30 s denaturation at 95°C, 45 s annealing at a temperature depending on the primer, 2 min primer extension at 72°C). The annealing temperature was 58°C for both GT ($YHY(GT)_7G$, where H = A/C/T and Y = A/C/G) and TCC primer ($YDD(TCC)_5$, where Y = A/C/G and D = A/G/T). The reaction was terminated with a 7 min extension at 72°C. Reaction products were resolved by electrophoresis on agarose gels containing 1.0% agarose (FMC BioProducts) and 1.0% SynerGel (Diversified Biotech). The lengths of the amplification products were estimated by comparing them to a 100 bp

Table 2. Variation of metric and meristic characteristics of *Andrya arctica* in three sampling locations. The last column shows the statistical difference in the mean values between locations. Results for pairwise tests in parentheses; statistically similar groups combined by '+'

	Yamal Peninsula (Y)				Western Kolyma (K)				Nunavut (N)				
Variable	*N*	Mean	SD	Range	*N*	Mean	SD	Range	*N*	Mean	SD	Range	Kruskall–Wallis test
Body length	3	106.0	37.3	74–123	7	112.9	16.0	90–136	9	94.0	16.0	74–123	NS
Scolex, diameter[1]	6	0.275	0.010	0.26–0.29	8	0.339	0.024	0.30–0.38	10	0.280	0.028	0.24–0.32	P=0.003 (Y+N,K)
Suckers, diameter[1]	5	0.154	0.006	0.150–0.165	8	0.166	0.008	0.155–0.180	10	0.159	0.012	0.140–0.180	NS
Testes number													
Total[1]	7	73.3	8.38	65–89	8	82.4	4.43	75–88	12	70.1	7.40	59–83	P=0.005 (Y+N,Y+K)
Antiporal	7	19.6	3.26	15–23	7	14.1	1.68	12–17	11	15.9	5.17	8–23	P=0.030 (Y+N,K+N)
Poral	7	6.0	1.82	3–8	7	6.6	1.27	4–8	11	3.7	2.53	0–8	P=0.036 (Y+K+N)
Mature segment, width	7	0.89	0.07	0.82–1.01	8	1.14	0.19	0.92–1.43	12	1.03	0.21	0.82–1.49	P=0.015 (Y+N,K+N)
Cirrus sac, max. length	7	0.483	0.035	0.43–0.53	8	0.034	0.034	0.40–0.50	12	0.449	0.034	0.40–0.50	NS
Seminal receptacle, max. length	7	0.519	0.080	0.40–0.61	8	0.657	0.120	0.40–0.80	12	0.610	0.127	0.45–0.90	NS
Unilateral segments[2]	11	2.94	0.71	2.3–4.8	7	2.42	0.30	1.82–2.74	10	2.62	0.17	2.42–2.90	NS

[1] Statistical test based on residuals from the regression between the width of mature segment and the particular measurement.
[2] Number of segments in a set of unilateral consecutive segments.

Table 3. Variables used in the principal component analysis and their loadings with the two main principal components (PC). Highest loadings in bold

Variables	Principal components PC1	PC2
Ovary, width[1]	0.095	**−0.385**
Ovary, length	**0.288**	−0.086
Vitellarium, width[1]	0.231	−0.280
Vitellarium, length	**0.260**	0.056
Cirrus sac, width	**0.303**	−0.094
Cirrus sac, length[1]	**0.302**	0.126
Internal seminal vesicle, width	**0.266**	−0.300
Internal seminal vesicle, length[1]	**0.274**	−0.099
External seminal vesicle, width	**0.305**	−0.008
External seminal vesicle, length[1]	**0.312**	0.089
Vagina, length	0.213	0.237
Seminal receptacle, width	**0.294**	0.025
Seminal receptacle[1], length	**0.304**	−0.105
Egg, width	−0.150	**−0.531**
Egg, length[1]	−0.146	**−0.531**

[1] Because of the significant correlation between the width of mature segments and the particular variable, size-adjusted values (residuals) were used in the analysis. The original values were used for the other variables.

DNA ladder (Gibco BRL). Electrophoreses were run in TAE-buffer (40 mM Tris-Acetate pH 8.0, 1 mM EDTA) and reaction products were detected by ethidium bromide staining.

CLONING OF RAMS FRAGMENTS FOR SEQUENCE TAGGED SITES (STS)

Two RAMS amplification products obtained with two different primers (*A. arctica* 580 bp TCC fragment and *A. arctica* 990 bp GT fragment) were chosen as suitable for development of specific STS primers. Amplification products obtained using GT and TCC primers were ligated into pCR/2.1 vector using the TOPO/TA Cloning Kit (Invitrogen). The resulting recombinant plasmids were used to transform *Escherichia coli* DH5α as recommended by the manufacturer. Recombinant colonies were identified by colour selection after overnight growth at 37°C on Luria-Bertani agar plates (Sambrook, Fritsch & Maniatis, 1989) containing 150 μg/ml of ampicillin and 27 μg/ml of both X-gal (5-bromo-4-chloro-3-indolyl β-D-galactoside, Promega) and IPTG (isopropylthio-β-D-galactoside). For screening of the inserts, the plasmids were isolated according to Birnboim & Doly (1979). The sought-after fragments were selected by PCR amplifications using TCC or GT primers according to the protocol described above. Prior to sequencing the plasmids were isolated using QIAGEN Spin Plasmid Kit (QIAGEN GmbH, Germany). Selected cloned inserts were sequenced by A.L.F. DNA sequencer (Pharmacia Biotech, Uppsala, Sweden) using M13 reverse and forward primers and Thermo Sequenase fluorescent-labelled primer cycle sequencing kit (Amersham Pharmacia Biotech, England). The primers designed based on these sequences (not shown) were as follows: for *A. arctica* 580 bp TCC-fragment forward primer 5′ TTC CTC TCA GCT TGG CTA CC 3′ and reverse primer 5′ TTC GCA GTT AAG TCA GCA TAG C 3′ (Sequence Tagged Site A = STS-A) and for *A. arctica* 990 bp GT-fragment, forward primer 5′ TTA CCT TCT CGG TTG GTC TCA 3′ and reverse primer 5′ AAT GGC CTA ACT TCA CCG C 3′ (Sequence Tagged Site B = STS-B). For efficient separation of the amplification products of the STSs in DGGE, a 41 bp long GC-clamp was added to STS-A-F and STS-B-R. Thus the primers used were: 5′ CGC CCG CGG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCT TCC TCT CAG CTT GGC TAC C 3′ (GC-clamped STS-A-F) , 5′ TTC GCA GTT AAG TCA GCA TAG C 3′ (STS-A-R), 5′ 5′ TTA CCT TCT CGG TTG GTC TCA 3′ (STS-B-F) and 5′ CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCA ATG GCC TAA CTT CAC CGC 3′ (GC-clamped STS-B-R).

STS ANALYSIS

Optimization of PCR conditions for the specific markers resulted in the following protocol: denaturation by 10 min incubation at 95°C after which 37 cycles of amplification were carried out (30 s denaturation at 95°C, 1 min annealing at 49°C for specific marker STS-A and 48°C for specific marker STS-B, 1 min primer extension at 72°C). The reaction was terminated with a 7 min extension at 72°C. The concentration of the primers was 1 μM. Amplification products were checked by electrophoresis on agarose gels as described above. The denaturing gradient gel electrophoresis was run according to the instructions of the manufacturer of the D-GENE denaturing gradient gel system (BioRad) using 7.5% (w/v) acrylamide/bisacrylamide (37.5:1) gels with a denaturant gradient of 25–40% for STS-A and 35–50% for STS-B. The 100% denaturing solution contained 40% deionized formamide and 7 M urea. Also these electrophoreses were run in TAE-buffer and reaction products were detected by ethidium bromide staining.

HIERARCHICAL ANALYSIS OF GENETIC DIVERSITY

The analysis of molecular variance was carried out using AMOVA software (Excoffier, Smouse & Quattro, 1992). Haplotypes for both STS-A and STS-B polymorphic loci were used, and every specimen listed in Table 1 was included in the analyses. The material

was divided into two main regions; the Palearctic and the Nearctic. Within the Palearctic the material was divided into three groups according to different chromosome races of the hosts (Fedorov *et al.*, 1999a); Group P1: Yamal and Taimyr, Group P2: Kolyma and Group P3: Wrangel Island. The Nearctic region was divided as follows; Group N1: Greenland, Group N2: Walker Bay, Hope Bay and Breakwater Island Nunavut, Canada, and Group N3: Victoria Island Nunavut, Canada (see Table 1 for groups). The wide strait separating Victoria Island from the mainland and the smaller islands justified the division of the Nunavut area into two groups. As this strait is approx. 30 km at its narrowest part, we addressed the possibility of a lack of recent gene flow between the two Nunavut populations (corresponding to our groups N2 and N3) in Canada.

In addition to the AMOVA analysis, also the coefficient of gene differentiation (G_{st}) was calculated according to Nei (1973). The G_{st} listed in the results table was calculated as an average of both markers studied.

MINISATELLITE SURVEY

DNA was amplified using the M13 core sequence as primer (Stenlid, Karlsson & Högberg, 1994). The reaction conditions for the PCR were the same as for the RAMS-primers described above. The cycling parameters were: 37 cycles of denaturing, 95°C 30 s, annealing, 48°C 1 min, extension, 72°C 2 min, final extension, 72°C 10 min. The amplified products were treated as the RAMS products and gel interpretations were made from photographic prints. Only clear and distinct bands were considered.

The analysis of genetic diversity of M13-RFLP fragments was carried out using AMOVA software and also Nei's coefficient of gene differentiation was calculated as an average of all markers (putative loci) studied. In these analyses the grouping was done as in the analyses of STS data described above.

RESULTS

MORPHOLOGY

According to Rausch (1952), the combination of morphological features that distinguish *Andrya arctica* from related species are irregularly alternating genital pores, very large cirrus sac and seminal receptacle, distribution of testes (antiporally and anteriorly to ovary), large eggs and completely reticulate early uterus. The last characteristic also serves as the main feature for the generic assignment of *A. arctica*.

With the exception of the material from Wrangel Island, all the specimens studied by us agree completely with the description of Rausch (1952), and have therefore been identified as *A. arctica* (Figs 2 and 3). Haukisalmi & Henttonen (2000) provided detailed morphological differences between *A. arctica* and *Paranoplocephala serrata*, the other anoplocephalid cestode described from collared lemmings.

The specimens from Wrangel Island were unique in two respects. Genital openings were strictly unilateral and no testes were situated on the poral side of the poral ventral osmoregulatory canal in any specimen (in other locations the mean number of poral testes ranged between 3.7–6.6) (Fig. 3, Table 2). However, the uterine development shows that the specimens from Wrangel Island also belong to *Andrya*, and they are therefore closely related with the more widespread taxon. The two specimens from Greenland did not differ morphologically from the rest of the specimens studied.

We also examined several more detailed morphological features, though none differed between the locations studied by us, including the Wrangel Island specimens. These were the structure of the cirrus sac (strongly developed musculature) and vagina (tube-like organ of uniform width), covering of the external seminal vesicle (dense layer of small 'prostatic' cells), distribution of testes (not overlapping ovary) and the position of vitellarium and ovary (median). The role of these characteristics in the taxonomy of *Andrya* and *Paranoplocephala* has been discussed by Genov, Vasileva & Georgiev (1996).

MORPHOMETRICS

Five of the ten univariate morphometric comparisons showed a statistically significant difference among the three locations in Table 2. According to the pairwise comparisons, the specimens from Yamal and Nunavut were indistinguishable in all five cases, the specimens from Kolyma and Nunavut in three cases, and those from Yamal and Kolyma in one case only. Univariate morphometric tests thus link better the Palearctic and Nearctic populations than the two Palearctic ones.

We considered two vectors (principal components) of the principal component analysis that had the highest eigenvalues (both >1). The first principal component (PC1) was responsible for 51.6% of the total variance and showed high loadings with most of the variables included in the analysis, excluding the egg dimensions (Table 3). The PC1 can thus be interpreted as a measure of general organ size in *A. arctica*. The PC2 explained 15.3% of the variance, and showed highest loadings with the egg dimensions and width of ovary. Both PC1 and PC2 were significantly different among the three locations (Table 3). Pairwise tests showed that PC1 was similar in Yamal and Nunavut, and both of them showed significantly lower values than the specimens from Kolyma (Fig. 4). PC2 showed two homogeneous groupings: Yamal + Kolyma and Kolyma + Nunavut.

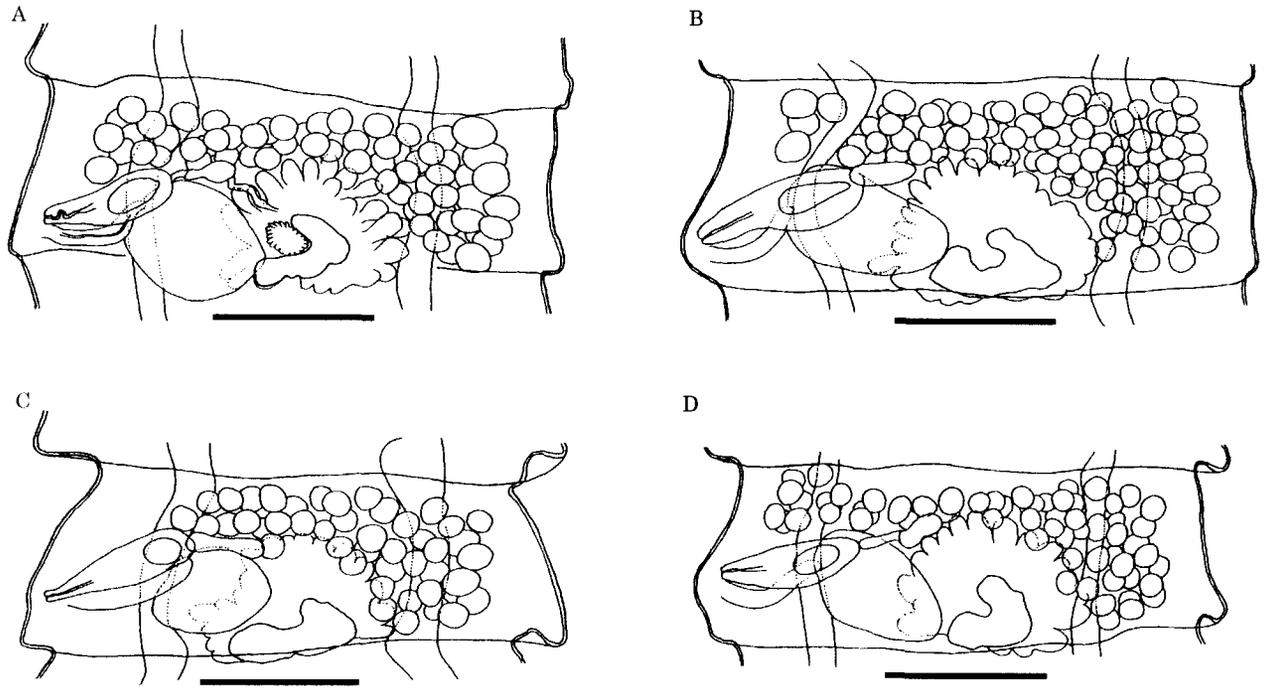

Figure 3. Representative mature segments of *Andrya arctica* from different locations. A, Yamal Peninsula (host *Dicrostonyx torquatus*); B, Western Kolyma Delta (host *Dicrostonyx torquatus*); C, Wrangel Island (host *Dicrostonyx groenlandicus*); D, Kent Peninsula region, Nunavut (host *Dicrostonyx groenlandicus*). Scale-bars: 0.30 mm.

RAMS-PATTERNS

The patterns of amplification products using GT primer showed substantial variation within *A. arctica* (not shown). With TCC primer, only two clear and reproducible bands were observed (not shown). The 580 bp TCC fragment and the 900 bp GT fragment were selected for development of specific STS markers due to the possible intraspecies length polymorphism observed in these fragments. The amplification product sizes expected with the designed primers were about 320 bp and 410 bp, respectively.

STS ANALYSIS OF ALLELIC VARIATION

For *A. arctica*, six alleles at the locus STS-A and five alleles at the locus STS-B were detected within the Holarctic region (Table 1). For the locus STS-A, the most common allele was STS-A2, which occurred in 18 of the specimens scored, ten of them being heterozygous. The allele STS-A5 was found in 13 specimens, five of which were heterozygous. Alleles four and six at locus STS-A occurred only once in the material. The most common allele for locus STS-B was STS-B3. Twenty specimens carrying this allele were scored, and seven of these were heterozygotes. All specimens in the survey carrying alleles STS-B2 or STS-B4 in locus STS-B were heterozygotes with B1 and B3, respectively. Twelve of the 30 specimens analysed in total were heterozygous for STS-A, 13 of 30 for STS-B and seven specimens of 30 were heterozygous for both STS-A and STS-B.

GEOGRAPHICAL DISTRIBUTION OF ALLELES

In the Palearctic, the differences in allelic composition between locations were more pronounced for STS-B, as all groups (Table 1) had varying allelic makeup in this locus (except for the one specimen from Taimyr that could not be distinguished from the Yamal specimens). The Yamal and Taimyr specimens could be identified by the presence of the alleles STS-B1, B2 and A2. These did not occur in any other Palearctic group. From the Kolyma River delta, only one allele for each locus was observed, namely STS-B3 and STS-A5. STS-B3 separated this group from the other Siberian groups. The specimens from Wrangel Island displayed two locality specific alleles, STS-B5 and STS-A6. These did not occur in any other studied specimen throughout the whole Holarctic region (Table 1).

As might be suspected from the distances between the survey sites, the Canadian groups were more alike than the Siberian groups. Considering locus STS-A, only Walker Bay (allele A4) and Victoria Island (allele A5) could be separated from other Canadian groups (alleles A1 and A2 occurred in all Canadian groups).

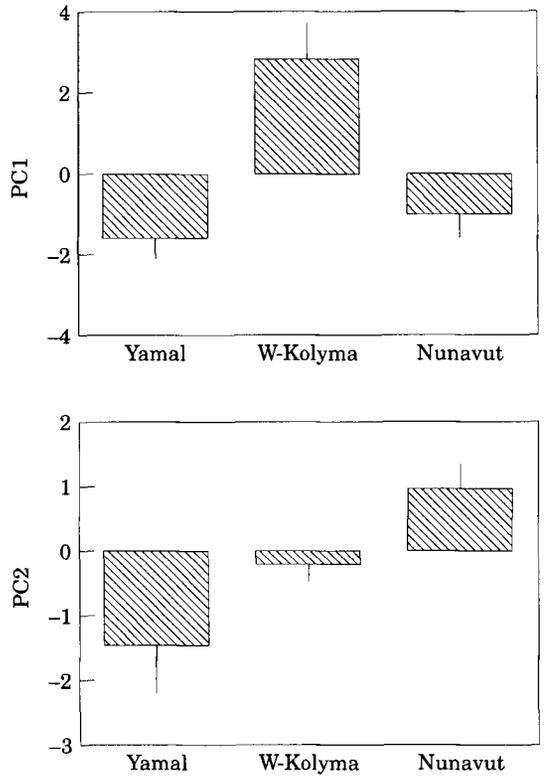

Figure 4. Mean values and standard errors of the mean for the two main principal components (PC) based on the dimensions of the reproductive organs and eggs of *Andrya arctica*. See text for statistical significances of differences between the three locations.

The majority of the Canadian specimens were homozygous for allele STS-B3 or STS-B3/B4 heterozygotes. In addition, the specimens from Greenland were STS-B3 homozygotes. Allele STS-A3, however, distinguished Greenland from all other localities. Comparing the Palearctic and the Nearctic, no major differences were seen in the allelic arrays, besides the absence of allele STS-B4 from the Palearctic and Greenland.

Hierarchical analysis of genetic diversity (Table 4; groups explained in materials and methods and depicted in Table 1) indicated that 12% of the genetic diversity was due to variation between the Palearctic and the Nearctic (Analysis 1, Table 4). However, this figure was not statistically significant ($P=0.16$). In the same test, the variance among all Holarctic groups was 46% ($P<0.0001$), and within groups 42% ($P<0.0001$). When analysing the Palearctic and Nearctic groups separately, the variance among groups in the Palearctic (Analysis 3, Table 4) was 73% ($P<0.001$) and within groups 27% ($P<0.0001$). The corresponding figures for the Nearctic (Analysis 4, Table 4) were 33% ($P< 0.0001$) between groups N1, N2 and N3, and 67% ($P<0.0001$) within groups. When excluding Greenland (N1) from the Nearctic (Analysis 5, Table 4), the variance among Canadian groups (N2 and N3) became even smaller (18%, $P=0.05$) which was expected as the geographical range of the sampling locations was restricted to approx. 125 km. Analysis 2 in Table 4 was done as a comparison to the level of genetic differentiation among all groups studied (Table 5). In this analysis the variance among all groups (when not divided into Palearctic and Nearctic regions) was 56% ($P<0.0001$) and within groups 44% ($P<0.0001$).

The level of genetic differentiation (Table 5) calculated according to Nei, resulted in high levels of differentiation among the Palearctic groups ($G_{st}=0.53$). When all groups studied were included the differentiation was equally high ($G_{st}=0.53$). The genetic differentiation among the Nearctic groups showed a lower level of differentiation ($G_{st}=0.26$) and when excluding Greenland (N1) and considering only the Canadian groups (N2 and N3), an even lower level of genetic differentiation was reached ($G_{st}=0.09$). For STS-A and STS-B the same general patterns were obtained both from AMOVA and G_{st} analyses.

VARIATION REVEALED BY AMPLIFICATION OF MINISATELLITE DNA

As the sample sizes of groups studied here are very small, we carried out a complementary analysis for the results obtained with STS markers. This test was done using M13 fingerprints, which are based on minisatellites. DNA was successfully amplified from all specimens in Table 1 using the M13 core sequence as primer. Eight to ten amplified fragments were scored from all other locations except from Greenland where only five and six clear bands were amplified. A distinct pattern of bands was recorded from Yamal, Taimyr, Kolyma and Wrangel, whereas the Canadian specimens showed variation even within the locations. The pattern for the M13 minisatellite primer basically shows the same result as the STS analysis suggesting that despite the small sample sizes the overall picture emerging is reliable. Differences are mainly recorded between groups in the Palearctic, whereas in the Nearctic the diversity is mainly recorded within groups. Of 18 scored fragments, 17 were shared by Palearctic and Nearctic groups and only one fragment ($\sim$530 bp), that could be found from the Nearctic specimens, was totally missing from the Palearctic (Fig. 5, Table 1).

Analysis of genetic diversity (Table 4) with AMOVA (Excoffier *et al.*, 1992) indicated that approximately 69% ($P<0.0001$) of the total genetic diversity (Analysis 1) was due to variation among all Holarctic groups (groups explained in Table 1), while the variance within groups was *c.* 18% ($P<0.0001$). The variance among the Palearctic and Nearctic regions was *c.* 13%, but

Table 4. Analysis of molecular variance (AMOVA) based on STS and M13 markers. Five analyses were carried out. Analysis 1 was carried out in three hierarchical levels (between regions N vs. P, within regions both among parasite groups and within parasite groups). The other analyses were carried out in only two hierarchical levels. Among vs. within all groups N+P (analysis 2), among vs. within Palearctic (P) groups (analysis 3), among vs. within Nearctic (N) groups (analysis 4) and among vs. within Canadian groups (analysis 5). For further details on grouping, see Table 1

	STS (microsatellite markers)				M13 (minisatellite markers)			
	Variance component	φ-statistics	Prop. of variance component	*P*-value*	Variance component	φ-statistics	Prop. of variance component	*P*-value
1. Among regions (N vs. P)	0.36	0.12	12%	0.1538	0.35	0.13	13%	0.2283
Among groups within regions (N+P)	1.35	0.52	46%	<0.0001	1.80	0.79	69%	<0.0001
Within groups (N+P)	1.24	0.58	42%	<0.0001	0.48	0.82	18%	<0.0001
2. Among groups (N+P)	1.57	0.56	56%	<0.0001	2.02	0.81	81%	<0.0001
Within groups (N+P)	1.24		44%		0.48		19%	
3. Among groups (P)	2.20	0.73	73%	<0.0001	2.72	1.00	100%	<0.0001
Within groups (P)	0.83		27%		0.00		0%	
4. Among groups (N)	0.74	0.33	33%	<0.0001	1.44	0.74	74%	<0.0001
Within groups (N)	1.49		67%		0.50		26%	
5. Among groups (Canadian)	0.34	0.17	18%	0.0467	0.07	0.13	13%	0.0887
Within groups (Canadian)	1.60		82%		0.44		87%	

* *P* = probability of obtaining equal or larger value determined by 9999 randomization of the treatments.

Table 5. Genetic differentiation (G_{st}) among groups counted as mean values for all markers studied in *Andrya arctica*. The grouping is equal to the grouping used in Table 4 (excluding the analysis in three hierarchical levels)

	Genetic differentiation G_{st}	
	STS	M13
Among all groups (6*)	0.53	0.74
Among all Palearctic groups (3)	0.53	0.38
Among all Nearctic groups (3)	0.26	0.47
Among Canadian groups only (2)	0.09	0.02

* Number of groups.

the differences were not statistically significant ($P=0.23$). As no variance was recorded within groups in the Palearctic (Analysis 3), the variance among groups was 100% ($P<0.0001$) in this region. The variance among all the Nearctic groups (Analysis 4) was 74% ($P<0.0001$), and when excluding Greenland (Analysis 5) the variance fell to 13%, and was not significant any more ($P=0.09$).

Considering the levels of genetic differentiation (Table 5) as a mean value of all markers included (putative loci), the highest level of genetic differentiation was reached among all groups studied ($G_{st}=0.74$). Because of the many shared fragments (Table 1) in the Palearctic groups, the G_{st} remained at 0.38 compared to a G_{st} of 0.53 for the STS-analysis. The specimens from Greenland with lots of markers differing from the Canadian specimens resulted in a marked raising of the genetic differentiation ($G_{st}=0.47$) compared with the G_{st} for only the Canadian specimens ($G_{st}=0.02$).

DISCUSSION

PHYLOGEOGRAPHY OF COLLARED LEMMINGS

It has been suggested that populations living in areas glaciated during the Pleistocene tend to be genetically less variable than populations in nonglaciated areas (Sage & Wolff, 1996; Hewitt, 1996). For the High Arctic genus *Dicrostonyx*, the matter seems to be somewhat different as the populations of collared lemmings were depressed during the warm periods of the interglacials. Palaeontological data as well as the present distribution of *Dicrostonyx* show that this northernmost genus of rodents evolved in tundra landscapes and was always restricted to cold and dry environment (Fedorov *et al.*, 1999a). Forest expansion during warm climatic events disrupted the range of collared lemmings (Sher, 1991) and the genetic variation observed in the phylogeographical groups might have resulted from divergence *in situ* following bottleneck events

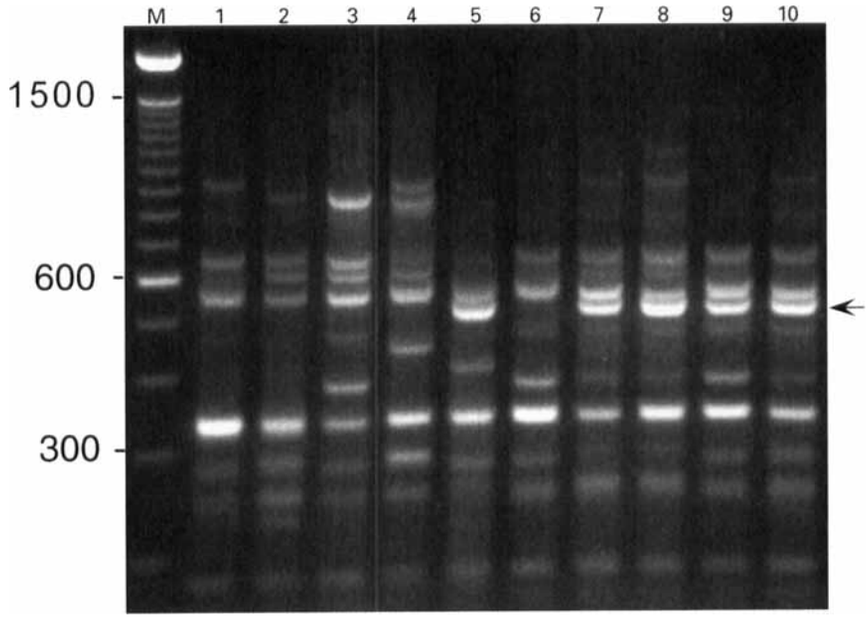

Figure 5. Banding patterns obtained with the M13 minisatellite primer. 1 – Yamal, 2 – Taimyr, 3 – Kolyma, 4 – Wrangel Island, 5 – Greenland, 6 and 7 – Walker Bay, 8 – Hope Bay, 9 – Byron Bay, 10 – Breakwater Island, M – fragment size marker (100 bp DNA ladder). The arrow indicates the ~530 bp fragment present only in Nearctic specimens.

during climatic warming of the Holocene. This hypothesis is also supported by the higher genetic diversity in the lemming populations from the area east of the Kolyma River and from Wrangel Island compared with western Siberian lemming populations. Eastern Kolyma was not affected by forest expansion during the Holocene (Khotinskiy, 1984) and Wrangel Island provided a relict of Pleistocene dry tundra in the Holocene (Vartanyan, Garutt & Sher, 1993). The variation in *D. groenlandicus* is more pronounced on Wrangel Island than in other locations probably due to the lack of bottlenecks and extinctions.

The main phylogenetic division in *Dicrostonyx* lies between the Eurasian *D. torquatus* and the North American group of species and is probably the result of intermittent inundation of the Bering Strait during the interglacials (Fedorov *et al.*, 1999a). However, Wrangel Island is considered to be colonized by lemmings of Nearctic origin (Fedorov *et al.*, 1999a). In North America the following vicariant separation in three refugial areas probably generated the extant species diversity (Fedorov *et al.*, 1999b). The amount of divergence among the three North American species suggests that the vicariant events predated the latest glaciation (Weichsel; 10–115 kyr; Andersen & Borns, 1997). *D. groenlandicus* evolved in ice free areas to the north of the main ice sheet, whereas the *D. hudsonius* and *D. richardsoni* likely derived from the southeastern and southwestern periglacial areas, respectively (Chaline, 1987; Engstrom *et al.*, 1993; Eger, 1995).

For *D. torquatus*, the phylogenetic grouping is due to historical population fragmentation and allopatric bottleneck events that divided the now continuously distributed species into five clades of haplotypes specific to different geographical regions within the Palearctic. The results of Fedorov *et al.* (1999a) suggest that during one of the last interglacials the distribution range of *D. torquatus* in Eurasia was contracted to a single refugium, probably situated to the east of the Kolyma river. The main phylogenetic split of *Dicrostonyx* is at the Bering Strait and corresponds to the main chromosome division between the Beringian and Eurasian groups of karyotypes. This suggests that despite the intermittent Bering land bridge, Palearctic and Nearctic forms have been separated since the mid Pleistocene *c.* 1 Myr ago (Fedorov & Goropashnaya, 1999).

GENETIC AND MORPHOMETRIC DIVERGENCE IN *ANDRYA ARCTICA*

The present results unequivocally show that the intestinal parasite *A. arctica* does not follow the major Palearctic–Nearctic phylogenetic split recorded for its host, *Dicrostonyx* (Fedorov *et al.*, 1999a). Morphometrics and all genetic comparisons show that the largest differences between *A. arctica* populations are found within the Palearctic region and not between the Palearctic and the Nearctic. In the Palearctic, three different genetic entities of *A. arctica* were found (Yamal, Taimyr and Kolyma) and two (Yamal, Kolyma) according to morphometrics. These regions correspond to the main chromosome races and phylogenetic groupings of the hosts (*D. torquatus*).

In principle, the lack of pronounced differences between the Palearctic and the Nearctic could be explained by long-distance dispersal of *A. arctica* by means of infected intermediate hosts (the intermediate host for anoplocephalid cestodes are soil arthropods, particularly oribatid mites; Gleason & Buckner, 1979; Denegri, 1993). However, this leaves open the question about differences in local divergence and degree of genetic variation in the Palearctic and Nearctic, respectively. Instead, the following explanation based on different evolutionary histories of *A. arctica* on the two continents would fit the observed patterns.

Originally a single genetically variable *A. arctica* population would have occupied the whole Holarctic area. Later the Palearctic and the Nearctic populations were separated from each other, and two equally variable parasite populations existed. This is supported by the fact that most of the genetic markers are observed both in the Nearctic and the Palearctic. After the separation, the Palearctic and the Nearctic host and parasite populations would have had different evolutionary histories. In the Palearctic, the populations would have gone through bottlenecks, which would have contributed to the low amount of within-population variation. This could have been due to contractions into several small refugia (random genetic drift acting within them) or by contraction to a single refugium and later dispersal events (resulting in the founder effect). This history in the Palearctic corresponds to the chromosome races and phylogenetic groupings of the hosts (*D. torquatus*) and suggests that the current groupings of the parasite may have the same origin as those of the host.

In the Nearctic, no severe population bottlenecks would have occurred, which would explain the high degree of within-population variation in *A. arctica*. The Nearctic thus contrasts with the history of the Palearctic in that there was no major spatial partitioning of the host and parasite populations within *D. groenlandicus*.

However, our sample from the Nearctic is restricted as all the parasite specimens came from a single host species (*D. groenlandicus*) and most of them from a single region. Samples from the northern and western Alaska, in particular, would be needed to assess the evolutionary history of *A. arctica* in the Nearctic. Interestingly, the holotype of *A. arctica*, which originates from North Alaska, was found to be morphologically

similar to the specimens from the Wrangel Island (unilateral genital pores, no testes poral to the poral ventral osmoregulatory canal). This could mean that there exists a Beringian form of *A. arctica* that is restricted to the western clade of *D. groenlandicus*, occurring in Alaska west of McKenzie River and on the Wrangel Island (Fedorov & Goropashnaya, 1999). The *A. arctica* populations on the Wrangel Island and in northern Alaska are morphologically and genetically so different from the Canadian and Siberian populations that these two taxa should be assigned to different species (Haukisalmi, Wickström, Hantula & Henttonen, submitted).

In the absence of phylogenetic hypothesis for different populations within *A. arctica* we can not determine plausibly whether the possible Beringian form of *A. arctica* has been derived from its wide-spread sister taxa by peripheral isolation or whether it is the ancestral form among the studied cestode populations. However, due to the restricted distribution of the 'Beringian form', we prefer the former possibility; this taxon would thus have originated in an isolated host population in eastern Beringia, possibly during the late Pleistocene (cf. Fedorov & Goropashnaya, 1999). We assume that the more widespread form of *A. arctica* was present in the precursor of all extant taxa of *Dicrostonyx*, and dispersed with its host to the Nearctic; this is supported by the presence of a morphologically identical form of *A. arctica* in *D. richardsoni* on the western coast of Hudson Bay (Rankin Inlet; Haukisalmi, Hoberg, Rausch & Beveridge, unpubl.). On the other hand, *A. arctica*-like cestodes have not yet been reported from the most divergent Nearctic representitive of *Dicrostonyx, D. hudsonius* (Schad, 1954).

Assuming that the host and parasite colonized Greenland postglacially from the ice-free areas of the Canadian Arctic islands (Fedorov & Goropashnaya, 1999), the genetic divergence of the Greenland population of *A. arctica* (host *D. groenlandicus*) can be explained by the founder effect (migration of a small number of individuals). It is also possible that collared lemmings 'overwintered' in the coastal part of North Greenland (Macpherson, 1965; Eger, 1995); in this case the parasite divergence would reflect genetic drift in a small refugial lemming population.

The genetical and morphological divergence of *A. arctica* suggests that the evolutionary history of this cestode species has included different processes acting on different geographical regions. In the Palearctic, the divergence of *A. arctica* has paralleled that of the hosts; this mechanism is analogous to the 'cospeciation' of host and parasite populations, although no taxonomical boundaries are recognized in Arctic Siberia. On the other hand, the similarity of most of the Palearctic and Nearctic populations is compatible with the concept of 'coadaption'; i.e. the parasite has remained relatively unmodified through a series of host speciation/divergence events because of large cohesive populations (cf. Brooks & McLennan, 1993). The uniqueness of the Greenland parasite population, and possibly also that of the Wrangel Island, can be explained by peripheral isolation, refugial effects or founder effects.

The present results contradict the pattern emerging from the studies on comparative biogeography of arctic marine homeotherms and their cestodes (Hoberg, 1992, 1995), a system in which the colonization of new host species by parasites in glacial refugia has been the principal mode of parasite speciation. However, the present study and those of Hoberg both show that the evolutionary history of host-parasite associations with broad geographical ranges are likely to be complex, and are seldom structured strictly by cospeciation processes.

ACKNOWLEDGEMENTS

The authors acknowledge V. Fedorov, K. Fredga, H. P. Gelter, G. Jarrell, C. J. Krebs and A. Kenney for supplying the lemming intestines, E. Vainio for some preliminary PCR tests, M.-L. Santanen and P. Gustafsson for skilful technical assistance, and E. P. Hoberg and I. Beveridge for helpful comments on the manuscript. We also thank R. L. Rausch for lending cestode material from his personal collection and J. R. Lichtenfels and P. Pilitt for lending the holotype of *Andrya arctica* from the US National Parasite Collection. This work was financed by the Academy of Finland (Research Council for Environment and Natural Resources).

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