J. Parasitol., 93(3), 2007, pp. 704–708
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Microsatellite Markers for the Human Nematode Parasite *Ascaris lumbricoides*: Development and Assessment of Utility

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ABSTRACT: We describe 35 microsatellite markers from the human parasitic nematode *Ascaris lumbricoides*. We found 7 sex-linked markers and demonstrate that 26 autosomal loci can be scored reliably. These markers have high genetic variability and provide the tools to address multiple questions concerning the epidemiology, fine-scale genetic structure, host specificity, and mating systems of this parasite.

Multiple codominant genetic markers provide the most versatile tools for investigating the population structure, mating systems, and host specificity of parasites. However, with the exception of a limited number of polymorphic allozyme loci (Anderson et al., 1993; Ibrahim et al., 1994) and single nucleotide polymorphisms (SNPs) within the introns of nuclear genes (Anderson and Jaenike, 1997), few easily scored codominant loci are available for *Ascaris* spp. Other marker types that have been used include dominant markers such as random amplified polymorphic DNA (RAPD) (Nadler et al., 1995), amplified fragment length polymorphisms (AFLP) (Nejsum, Freydenberg et al., 2005; Nejsum, Parker, et al. 2005), and sequence data from the mitochondrial DNA (mtDNA) or the internal transcribed spacers (ITS) of ribosomal DNA (rDNA) (Anderson et al., 1993, 1995; Peng et al., 1998, 2003, 2005). These markers have provided important insights, but have several limitations. First, for example, population inferences based on mtDNA alone may be misleading, due to processes such as incomplete lineage sorting and introgression via hybridization (Anderson and Jaenike, 1997; Anderson, 2001; Ballard and Whitlock, 2004) or natural selection (Ballard and Whitlock, 2004; Hurst and Jiggins, 2005; Bazin et al., 2006). Second, the low polymorphism and presence of variation both within repeat arrays and between chromosomes complicates interpretation of rDNA ITS data (Blouin, 2002; Vilas et al., 2005). Third, dominant markers (RAPDs and AFLPs) preclude identification of heterozygotes, so deviations from Hardy–Weinberg equilibrium cannot be tested. This latter caveat is important because there may be fitness costs to being inbred (e.g., Christen et al., 2002) and because the change in allele frequency due to directional selection, e.g., antiparasitic drugs, is more rapid in inbred populations (Hedrick, 2005a). Furthermore, sex-linked and autosomal RAPD or AFLP markers cannot be easily differentiated. Treating sex-linked markers as autosomal loci will bias estimates of allele frequencies and can result in incorrect inferences about the genetic structure among populations. Finally, dominant markers are of limited utility in determining mating systems because alleles from both parents cannot be visualized.

The high allelic polymorphism commonly exhibited by microsatellite markers (1–6 bp tandem repeats of DNA sequence) and the ability to genotype individuals at many microsatellite loci allow these markers to be used in a variety of population genetic studies. Such applications include the estimation of inbreeding, migration, relatedness, parentage, effective population size, hybridization, and population assignment (Jarne and Lagoda, 1996; Luikart and England, 1999); and the investigation of mating systems or transmission patterns of parasites among hosts (e.g., Criscione et al., 2005; Criscione and Blouin, 2006). Despite their potential utility, only 5 microsatellite primer pairs have been published to date for *Ascaris* spp. (Anderson et al., 2003).

Here, we present a set of 35 microsatellite markers developed from *A. lumbricoides*. Our objectives in this study were (1) to determine the genotyping reliability of these loci; (2) to characterize the patterns and levels of genetic diversity; and (3) to assess the utility of these loci in detecting roundworm genetic structure among individual human hosts, i.e., infrapopulations (Bush et al., 1997).

The 35 microsatellite loci reported in Table I were generated from 3 sources. We designed primers for 2 sequences that were in GenBank (AF205422 and AF205424). Five primer pairs were designed from sequences obtained from the *A. suum* EST database on Nematode.net (Table I). The remaining 28 were generated from 2 genomic libraries. The 1st library was generated from the combined extracted DNA of 5 Bangladeshi and 5 Guatemalan female worms and the 2nd library was from 4 female Nepali worms. All worms were obtained from human hosts by the methods described in Williams-Blangero et al. (1999, 2002). Protocols for DNA extraction are given in Anderson et al. (2003). Construction of the libraries followed Hamilton et al. (1999). The following biotinylated oligos were used to enrich the libraries: AC_{15}, AG_{15}, $ACCT_{15}$, $AAAC_{15}$, and AAG_{15}. Bacterial colonies were screened with the use of the Phototope-Star Detection Kit from New England Biolabs, Inc. (Ipswich, Massachusetts). Positive colonies were scraped with a pipette tip, which was subsequently immersed in sterile water. These colonies were boiled and then used directly as template for 25-μl PCR reactions (1× PCR buffer, 0.25 mM dNTP, 0.4 μM each primer, 0.25 U TaKaRa Taq™ (Takara Shuzo Co., Otsu, Shiga, Japan), 2.5 mM $MgCl_2$, 1 μl template DNA), with T3 (AATTAACCCTCACTAAAGGG) and T7 (TAATACGACTCACTATAGGG) primers. PCR products were directly sequenced in both directions with the use of a BigDye 3.0 dye terminator sequencing kit (Applied Biosystems, Foster City, California) and T3 and T7 primers. Sequence reaction products were then electrophoresed on an ABI 3100 capillary sequencer. Forward and reverse sequences were compared and aligned with the use of Sequencher V4.2 (Gene Codes Corp., Ann Arbor, Michigan). Oligos for all loci were designed using PRIMER3 (Rozen and Skaletsky, 2000). All primers were ordered from ABI with the tailing option in order to reduce any potential polyadenylation effects.

We extracted DNA from individual worms as reported previously (Anderson et al., 2003). With female roundworms, we took special care to avoid tissue that potentially can have allelic contamination from male sperm, e.g., uterus (Anderson et al., 2003). Furthermore, any individuals (less than 5% of the data set) that showed 3 or more alleles at any single locus were excluded. Some individuals that had multiple alleles were reextracted. Subsequent PCR showed only 1 or 2 alleles at the suspect locus, thus confirming that sperm contamination was the likely cause (see also Anderson et al., 2003). The PCR reaction mix was as reported in Anderson et al. (2003), but the total volume was scaled down to 5 μl and the final concentration of $MgCl_2$ was 2.5 mM. We used a ramp-down PCR cycling method. There was an initial denaturing step (96 C for 5 min), followed by 5 cycles of high-temperature PCR (96 C for 45 sec, 55 C for 30 sec, 72 C for 1 min), then 35 cycles of low-temperature PCR (96 C for 45 sec, 47 C for 30 sec, 72 C for 1 min), and a final step of 72 C for 7 min. Loci for which cycling conditions varied are shown in Table I. PCR and genotyping error were assessed by duplicating PCRs on a subset of 20 individuals for all loci.

We screened 108 adult *A. lumbricoides* (61 females and 47 males) from 38 human hosts who resided in a single village in Jiri, Nepal, to determine patterns and levels of genetic diversity (Table I). Observed heterozygosity (H_O) and gene diversity (H_E) at each locus were calculated in GENEPOP version 3.4 (Raymond and Rousset, 1995). We found between 2 and 31 alleles per locus, and H_E ranged from 0.12 to 0.95 (Table I). However, for 7 loci (ALAC01, ALGA24, ALGA40,

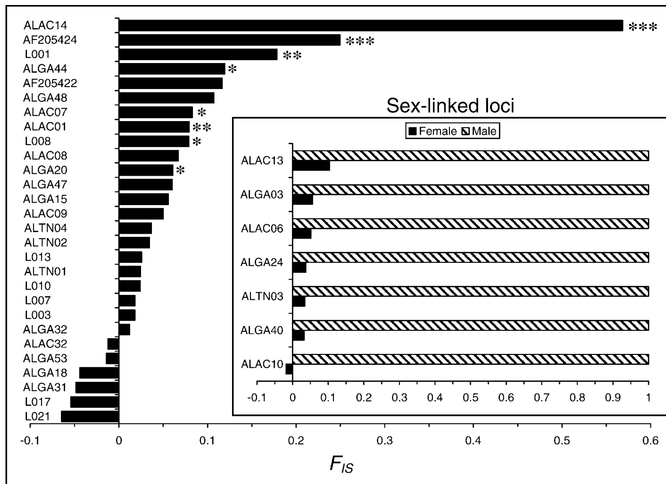

FIGURE 1. F_{IS} for each locus. Negative values indicate heterozygous excess. Positive values indicate homozygous excess. Significance from Hardy–Weinberg equilibrium was determined by permutations of alleles among individuals (*0.05 ≥ P > 0.01, **0.01 ≥ P > 0.001, ***0.001 ≥ P). Inset shows sex-linked loci.

L010, L013, AF205422, and AF205424), the number of alleles is not the true total. Microsatellite stutter for these 7 loci increased in the larger alleles. Therefore, we binned large alleles into a single class for these loci to avoid ambiguity in scoring. PCRs on the duplicate samples always produced the same peaks except for locus AF205424, where the large allele peak heights varied and sometimes failed between duplicates. Therefore, we expected that AF205424 would have an elevated F_{IS} as a result of large allele dropout. In addition, ALAC14 displayed an unusual pattern, where rare alleles were present as homozygotes or with other rare alleles as heterozygotes, but almost never with the single predominate allele (frequency = 0.92). Thus, we also expected an elevated F_{IS} with ALAC 14.

We calculated the Weir and Cockerham (1984) estimator of F_{IS} for each locus with the use of SPAGEDI V1.2 (Hardy and Vekemans, 2002). Positive values of F_{IS} indicate an excess of homozygotes, whereas negative values indicate an excess of heterozygotes from that expected under Hardy–Weinberg equilibrium. We tested for deviations from 0, i.e., a test of Hardy–Weinberg equilibrium, at each locus (and for the multilocus estimate) by randomizing alleles among individuals 20,000 times (Fig. 1). We concluded that 7 loci (ALAC06, ALAC10, ALAC13, ALGA03, ALGA24, ALGA40, and ALTN03) were sex linked because these loci were homozygous in all males (F_{IS} = 1), but did not deviate from Hardy–Weinberg equilibrium in female worms (Fig. 1). If sperm contamination was a problem, we would expect that females would be more heterozygous, i.e., have lower F_{IS}, than males across the autosomal loci. However, males had lower F_{IS} in 18 of the 28 autosomal loci, which was not significant (sign test, P = 0.186) (data not shown). Of the 28 autosomal loci when males and females were pooled, 8 showed significant ($P \leq 0.05$) heterozygote deficit (Fig. 1). However, only ALAC14 and AF205424 remained significant after sequential Bonferroni correction for multiple testing (Rice, 1989). Furthermore, these were the only 2 autosomal loci to produce significant within host values of F_{IS} in a hierarchical analysis (see below) after sequential Bonferroni correction (data not shown). These results confirmed our a priori expectations that both ALAC 14 and AF205424 would deviate from Hardy–Weinberg equilibrium. Therefore, we considered these 2 loci as outliers and excluded them from all subsequent analyses. In the case of ALAC14, we cannot discriminate between different possible explanations of this pattern, e.g., null alleles, presence of a duplicated locus, or selection; however, it is clear that the F_{IS} of this locus falls well outside the range of values reported for the remaining autosomal loci (Fig. 1). The elevated F_{IS} at locus AF205424 appeared to result from large allele dropout and/or null alleles. The multilocus estimate of F_{IS} based on 26 autosomal loci (excludes ALAC14 and AF205424) was 0.047, demonstrating significant heterozygote deficit ($P < 0.0001$).

The heterozygote deficit detected could result from nonrandom mating within hosts, and/or subdivision between parasites from different hosts, i.e., Wahlund effects. We used a hierarchical analysis of genetic structure (Weir and Cockerham, 1984) to differentiate between these explanations (Table II). For this analysis, we excluded hosts with fewer than 4 genotyped worms, reducing the data set to 66 roundworms from 7 people. We measured both F_{IS} (average within hosts) and F_{ST} (a measure of genetic differentiation among nematode infrapopulations) with the use of FSTAT V2.9.3 (Goudet, 1995). We tested the significance of the average F_{IS} within hosts with the use of 15,000 permutations of alleles among roundworms within hosts. We tested the significance of F_{ST} by the G-based test (Goudet et al., 1996) with 15,000 permutations of individual parasites among hosts. We also determined if the average relatedness (R) (Queller and Goodnight, 1989) among individual roundworms within hosts was significantly different from that expected with random parasite recruitment. For this test, we permuted individuals among hosts 10,000 times with the use of the program SPAGEDI V1.2. We found no significant deviations from Hardy–Weinberg equilibrium within hosts, although there was significant structure among hosts (F_{ST} > 0, Table II). These results indicate that the overall F_{IS} observed in our original analysis is caused by the admixture of parasite populations from separate infrapopulations, i.e., a Wahlund effect, rather than nonrandom mating within hosts. Note that the overall F_{IS} (0.047) estimated from the full data set above is approximately equivalent to the F_{IT} (0.041) of this hierarchical analysis, thus confirming that the reduced data set for the hierarchical analysis (66 worms) is representative of the full data that had 108 worms. Furthermore, the average relatedness of roundworms within hosts was greater than expected from a random recruitment of parasites among hosts (Table II). These patterns indicate that hosts may be sampling different source populations of parasites. Thus, the loci we developed appear to be useful in detecting fine scale patterns of genetic structure and parasite transmission.

We tested for genotypic disequilibrium between pairs of loci with the use of GENEPOP (Markov chain parameters: 5,000 dememorizations; 5,000 batches; 5,000 iterations) and used a sequential Bonferroni method to correct for multiple tests. We carried out genotypic disequilibrium tests for the 26 autosomal loci (excluding ALAC14 and AF205424) with combined males and females. We also did these tests with just females across all 33 loci (excluding ALAC14 and AF205424) so as to include the sex-linked loci. After sequential Bonferroni, there were no significant associations between pairs of loci.

Of the 35 microsatellite loci we developed for *A. lumbricoides* (Table I), 2 (AF205424 and ALAC14) are not likely to be useful in future studies. The utility of L021 may also be limited because it has only 2 alleles with a frequency of 0.94 for the common allele. The remaining loci all have substantial genetic variation and, therefore, will be useful for population genetic studies. Preliminary tests (data not shown) also show that all loci will amplify in roundworms obtained from pigs. We note that many of the loci also showed allelic variation due to indels, thus indicating that strict stepwise mutation models are not appropriate for these loci. Caution is needed when comparing levels of microsatellite genetic diversity among species because of differences in isolation protocols and repeat array length of the loci. However, the levels of gene diversity we find in *A. lumbricoides* are similar to that reported in a handful of other parasitic nematodes of animals (reviewed in Johnson et al., 2006). Microsatellites from trichostrongylid nematodes are frequently associated with a ~150-bp repeat element (TcREP-class of repeats) (Grillo et al., 2006; Johnson et al., 2006). We searched our library of clone sequences for homology to the TcREP repeat, but found no BLAST matches.

An interesting result from our microsatellite development was the finding of 7 sex-linked markers. These markers may be useful to help identify the sex of larvae or immature worms. It is noteworthy that *A. suum* has 19 autosomes and 5X chromosomes (2n = 38A + 10X in females, 38A + 5X in males) (Muller and Tobler, 2000). Thus, sex-linked markers should account for ~21% of the genome (assuming all chromosomes are of equal length). In accordance with this estimate, 20% (7 of 35) of our developed markers were sex linked. Interestingly, Johnson et al. (2006) found 3 of 21 (14%) sex-linked microsatellites from *Trichostrongylus tenuis*, which is assumed to have XO sex determination. If similar proportions (14–20%) of dominant markers are lo-

TABLE I. Microsatellite information and measures of genetic diversity (H_O, observed heterozygosity; H_E, gene diversity) from 61 female and 47 male *Ascaris lumbricoides*. Sex-linked markers are in bold.

Locus	GenBank accession	Primers*	Clone size (bp)	Motif	Number of alleles	H_O	H_E
ALAC01	DQ988845	[F]TGCCGGAATTTTATCTTCAA TGATCGACTGTCTATGCAAACC	246	(AC)n	26¶	0.870	0.945
ALAC06†‡	DQ988846	[P]AAAAACATGTGGCTTTGAAT GTCAGTGGTATAGGGCACAT	185	(GT)n	5	0.328	0.345
ALAC07	DQ988847	[P]AATCGTGTTCTTTGAAGTGG AACACGCTGAAATTGAAACT	233	(GT)n	18	0.759	0.828
ALAC08‡§‖	DQ988848	[V]CATTAATTGCAAAGCACAGA CGATTTTGCTGGCTATAGTT	312	(AC)n	25	0.759	0.814
ALAC09	DQ988849	[V]TGCAAATTTTACTATTTTAGCGTTT GATAATTTCATGCCCTACTTGAG	200	(GT)n	14	0.731	0.770
ALAC10†‖	DQ988850	[F]AGATTACGATGTGGCTGACT CGGAACAAATACAAATCCTC	128	(AC)n	5	0.508	0.499
ALAC13†	DQ988851	[F]CTGCAGTCGCGAAAAAGAA TGAAATTCTGTTTCATTCTCAAAA	139	(GT)n	9	0.656	0.731
ALAC14	DQ988852	[P]ACTGAGCAAGGATGCATGTG CAAATGCGAATATGCATGAAA	143	(AC)n	6	0.065	0.150
ALAC32	DQ988853	[F]AACGCTGCCACACCAGTATC ACCTGCGCACATCAAGAC	129	(GT)n	20	0.833	0.823
ALGA03†‖	DQ988854	[V]GCGTGAATGGATCATATTTC CGATAATGCAGATTTGTTGA	196	(TC)n	12	0.639	0.677
ALGA15‡§	DQ988855	[F]TTGGATGTTCTCATCTCATCTC ATGAGGAGYCATCACATTTT	306	(GA)n	15	0.824	0.873
ALGA18	DQ988856	[P]GATGCTGAGGAACAACAGAA TTTCAAGAAGAAGGAAAGTGG	225	(GA)n	9	0.157	0.151
ALGA20	DQ988857	[F]ATTGTTCCGTTGCTGGAAAG TATACCCACCCTTTCGCCTA	234	(GA)n	31	0.880	0.937
ALGA24†	DQ988858	[F]CGAATCAGAGAATGTTTAGCAA AAATGGTKGAATGTGAGAATTT	206	(TC)n	19¶	0.885	0.919
ALGA31	DQ988859	[P]CGCTTTCTTTTAATAACGCATA TGATGCATAAAAAGAAGTGATT	297	(TC)n	16	0.852	0.813
ALGA32	DQ988860	[V]CGTTCGCTCTAAAGAAATCA AAAATAAACAACAGCCTTCCA	184	(TC)n	23	0.889	0.900
ALGA40†‡	DQ988861	[N]AATTGCATCAAATCCTGACA TATTGGTGAGAAGGCGCAAG	283	(GA)n	12¶	0.705	0.728
ALGA44	DQ988862	[P]GCTGGAGACGCAACTAGATA CAACCAATTTTTGCAGATCA	220	(GA)n	9	0.639	0.725
ALGA47‡	DQ988863	[N]AAGTTGGGCTATTTTCCACA AAACGACAATGAACGGAAAT	171	(GA)n	20	0.843	0.896
ALGA48	DQ988864	[N]AGAAATTTTCCAACCGTTTTT TTGTGTTGGTTCCTCATTTG	218	(CT)n(CA)n	12	0.667	0.746
ALGA53	DQ988865	[F]GCGTTGACTAACATAGAGAAAT TGTGAGAATTAATGGGTTGC	217	(GA)n	17	0.324	0.320
ALTN01‡	DQ988866	[P]CGTGACACTCAGACAAATCA TGTTAATTCGATGAAAATGC	240	(CAAA)n	15	0.796	0.816
ALTN02	DQ988867	[N]CAAAACGGCACTATGAAAA ATATCGATTGGCTCATCAAC	249	(TTTG)n	10	0.546	0.566
ALTN03†	DQ988868	[N]TACCACTGAGCAGTCGCATC TACTATTCCGGGGATTGCAT	270	(AC)n(AAAC)n	14	0.787	0.815
ALTN04	DQ988869	[N]TAATTAGTGACAGCCGGAGT TTGGTCGCAAGAGTTAGAAT	193	(TTTG)n	13	0.722	0.750
L007	DQ988870	[P]TAAAAATCAATGCATCAACG AGAGTTTGCATCGAATTTGT	183	(AC)n	10	0.713	0.726
L008‡§	DQ988871	[P]GAGCAGCAATGTTCACTGTA TGGAAAAAATATCACGGAAGT	228	(TC)n	19	0.769	0.834
L010	DQ988872	[N]AACGTAATTTTCATGCTGCT AGGACTTGTTTTGACAGTGG	222	(GA)n	19¶	0.870	0.892
L001-est	CB101754 (kl60g02.y1) #	[V]TTCACAGTTGCTGTTTCTTG AGAGAACGTTTCTTATTTCAGC	99	(TA)n	5	0.417	0.507
L003-est‡§	BQ380931 (kk25a05.y1) #	[V]CGACATTTGTCTCTTCGTTT CTCGACACCACATACATCAA	99	(TA)n	4	0.296	0.302

TABLE I. Continued.

Locus	GenBank accession	Primers*	Clone size (bp)	Motif	Number of alleles	H_O	H_E
L013-est‡§	BM033372 (kh55a04.y1) #	[P]GCATAACCGCTGAAGATACT CGAACTGATAACTAGCAGAGAA	189	(TAT)n	10¶	0.769	0.789
L017-est	BQ835581 (kk63e09.y1) #	[F]TGTTTTGAGGTGGTTTTTCT TCATAGGGATGCTTAATGCT	365	(TTGA)n	5	0.380	0.360
L021-est	CB101812 (kl73f08.y1) #	[N]CGGATTGTATGCTCTCTTCT ATCAATTTTTCAATCGGCTA	276	(TTTA)n	2	0.130	0.122
AF205422	AF205422	[F]GACCGCACTGACTTTACAAC CGATGGATAAGATTTTCGTC	245	(AC)n	16¶	0.444	0.503
AF205424	AF205424	[V]GTATCGTCCGCTTAAAAACC ATGGTTTCTCCATCTGGTA	291	(CT)n	19¶	0.648	0.863

* Superscript at the 5′ end of the forward primer shows fluorescent label (F is 6FAM, P is PET, V is VIC, N is NED).
† Sex-linked loci. The number of alleles, H_O, and H_E are based on the 61 females only.
‡ Number of cycles was 38 in the 2nd cycling round.
§ Annealing temperatures were 53 and 45 C.
‖ Reported in Anderson et al. (2003). The forward primer for ALGA03 has been altered by 1 bp on the 5′ end.
EST sample name from Nematode.net Genome Sequencing Center.
¶ Fragments larger than a given size were binned into a single allelic range to avoid stutter-induced ambiguity in genotype scoring.

TABLE II. Genetic structure within (F_{IS}) and among hosts (F_{ST}), and average relatedness (R) within hosts.

	Estimate	Significance
F_{IS} (average within host)	0.015	Not significant
F_{ST} among hosts	0.027 (0.081)*	$P < 0.0001$
R within hosts	0.023	$P < 0.0001$

* Standardized F_{ST} is shown in parentheses. We standardized the Weir and Cockerham (1984) estimate of F_{ST} among infrapopulations by dividing the $F_{ST\text{-estimate}}$ by $F_{ST\text{-max}}$. $F_{ST\text{-max}}$ was calculated by recoding the data to obtain maximum divergence among populations, i.e., no shared alleles among populations (Meirmans, 2006). Within-population heterozygosity can be high with variable loci such as microsatellites; thus the maximum F_{ST} among populations may be much less than 1 (Hedrick, 2005b). Standardization corrects estimates of F_{ST} from multiallelic loci so they are comparable with estimates derived from diallelic loci (Hedrick, 2005b).

cated on the X chromosomes, then analysis methods using dominant markers will be subject to bias. For example, genetic structure studies with dominant markers rely on the assumption of Hardy–Weinberg equilibrium within populations to estimate heterozygote frequencies in the calculation of F_{ST}. However, males will be haploid for the locus under question; thus allele frequency estimates will be incorrect. Therefore, caution is needed when using dominant markers in *Ascaris* spp. and possibly other nematode parasites.

This work was funded by the National Institutes of Health under grant R01 AI037091 to Sarah Williams-Blangero. The molecular work at the Southwest Foundation for Biomedical Research was conducted in facilities constructed with support from Research Facilities Improvement Program Grant Number C06 RR013556 from the National Center for Research Resources, National Institutes of Health. We thank the field staff at the Jiri Helminth Project in Nepal for their assistance, and the Jirel people for their generous participation in the project. Protocols for this research were approved by the University of Texas Health Science Center Institutional Review Board in San Antonio, Texas, and by the Nepal Health Research Council, Kathmandu, Nepal.

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J. Parasitol., 93(3), 2007, pp. 708–709
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Abnormal Morphology of an Adult Rocky Mountain Wood Tick, *Dermacentor andersoni* (Acari: Ixodidae)

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ABSTRACT: During a collection of ticks from vegetation in March 2006, a single adult male Rocky Mountain wood tick, *Dermacentor andersoni* (Stiles, 1908), was collected that exhibited unique morphological anomalies, including the absence of a leg on the right side of the body. Coxa IV on the right side also was missing in this specimen. Such teratological changes have not been reported previously for *D. andersoni*.

Several studies have reported cases of morphological abnormalities in several species of ixodid ticks. The most frequent teratological changes are gynandromorphs, i.e., adult ticks exhibiting a combination of male and female morphological characteristics that have arisen as a consequence of the elimination of a maternal sex chromosome (X) from the zygote during embryonic development (Campana-Rouget, 1959a, 1959b; Oliver and Delfin, 1967; Homsher and Yunker, 1981; Labruna et al., 2002). There are, however, a variety of other types of teratological changes, including asymmetrical duplication of reproductive structures and structural deformities of the hypostome, palps, basis capitulum, idiosoma, and legs (Campana-Rouget, 1959b; Sakla et al., 1980; Latif et al., 1988; Buczek et al., 1991; Buczek, 2000; Estrada-Peña, 2001). In a review of the teratology of ticks, Campana-Rouget (1959b) separated the morphological abnormalities of appendages into 2 major categories: schizomélies, e.g., division of the claws tarsus and femur; and meiomélies, the latter of which were further divided into symmélies (fusion of appendages), atrophiés (appendages reduced in size), and ectromélies (the loss of 1 or more legs). The principal causes of morphological abnormalities in ixodid ticks are thought to be somatic or germinal mutations, exposure to chemical agents or environmental stress, and feeding on unusual or sensitized hosts (Campana-Rouget, 1959b; Sakla et al., 1980; Latif et al., 1988; Buczek, 2000). In some instances, teratological changes in ticks have been induced experimentally (Campana-Rouget, 1959b; Oliver and Delfin, 1967; Buczek, 2000). Nonetheless, the occurrence of morphological anomalies in field-collected ticks is relatively infrequent (Sakla et al., 1980; Tovornik, 1987; Guglielmone et al., 1999; Labruna et al., 2002). In this study, we report the discovery of an anomaly in the morphology of an adult male Rocky Mountain wood tick, *Dermacentor andersoni*.