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A preliminary linkage map of the tick, *Ixodes scapularis*

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Abstract. A linkage map of the *Ixodes scapularis* genome was constructed based upon segregation amongst 127 loci. These included 84 random amplified polymorphic DNA (RAPD) markers, 32 Sequence-Tagged RAPD (STAR) markers, 5 cDNAs, and 5 microsatellites in 232 F₁ intercross progeny from a single, field-collected P₁ female. A preliminary linkage map of 616 cM was generated across 14 linkage groups with one marker every 10.8 cM. Assuming a genome size of $\sim 10^9$ bp, the relationship of physical to genetic distance is ~ 300 kb/cM in the *I. scapularis* genome.

Key words: *Ixodes scapularis*, linkage map, microsatellites, RAPD-SSCP, STARs, cDNA-SSCP

Introduction

Ticks and the pathogens they transmit have long been an important cause of morbidity and mortality. Ticks are second only to mosquitoes as vectors of human and animal diseases (Sonenshine, 1991). *Ixodes scapularis* has become an important vector species because of its ability to transmit *Borrelia burgdorferi*, the causative agent of Lyme disease, in eastern and midwestern North America (Spielman *et al.*, 1985). *I. scapularis* is also a vector of the causative agent of human granulocytic ehrlichiosis (HGE) (Telford *et al.*, 1996) and *Babesia microti* the causative agent of human babesiosis (Spielman, 1976).

A great deal of information is known about the development, physiology, anatomy and vector competence of ticks, however little is understood about the genetic basis of these traits. Karyotypes have been characterized for 103

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of the ~830 known tick species (Oliver, 1977). However, a linkage map has never been produced for any of these or, for that matter, for any chelicerate arthropod. This is in part due to the minimal generation time required to establish a tick mapping family, which, at a minimum, over 1 year and 10 months are required.

Once an F₁ intercross family has been established, the modern paradigm for linkage mapping involves simultaneous estimates of recombination amongst hundreds (Antolin *et al.*, 1996) or potentially thousands (Yasukochi, 1998) of highly polymorphic loci. This typically yields an intensive linkage map with one marker distributed every 5 cM. Many different genetic markers are currently in use for intensive linkage mapping. One class includes simple sequence repeats or microsatellites (MS). MS-based linkage maps have been generated for anopheline mosquitoes (Zheng *et al.*, 1996), mice (Dietrich *et al.*, 1992) and humans (Hudson *et al.*, 1995). Characterization of MS markers in *I. scapularis* has been described (Fagerberg *et al.*, 2001) but the abundance of and heterozygosity at MS loci was generally low.

Random Amplified Polymorphic DNA amplified by the Polymerase Chain Reaction (RAPD-PCR) provides another class of highly polymorphic markers. This technique uses a short (10 oligonucleotide) primer to amplify small regions of a genome (Williams *et al.*, 1990). Alleles amplified by RAPD-PCR are typically dominant. Recessive alleles arise through mutations in or around the primer annealing site that cause the PCR process to fail. Genetic linkage maps utilizing RAPDs have been created for many insects including *Anopheles gambiae* (Dimopoulos *et al.*, 1996), *Aedes aegypti* and a parasitic wasp, *Bracon hebetor* (Antolin *et al.*, 1996), *Apis mellifera* (Hunt and Page, 1995), and *Bombyx mori* (Yasukochi, 1998). However, a great deal of the information needed to estimate recombination frequencies is lost when mapping loci with dominant alleles.

Single strand conformation polymorphism (SSCP) analysis of RAPD markers can reveal alternative, codominant alleles (Antolin *et al.*, 1996). SSCP analysis is based on the principle that electrophoretic mobilities of single-strand DNA molecules in nondenaturing gels are dependent upon both the size and shape of the fragments. Several stable structures or conformations can be formed when secondary base pairing occurs among nucleotides on a single DNA strand. The SSCP technique detects 99–100% of point mutations in DNA molecules 100–300 base pairs (bp) in length and at least 89% of mutations in molecules 300–450 bp in length (Orita *et al.*, 1989; Hayashi, 1991; Hiss *et al.*, 1994; Vidal-Puig and Moller, 1994).

Sequence-Tagged RAPD loci (STARs) (Bosio *et al.*, 2000) are another useful modification of RAPD markers. A RAPD-PCR band from a SSCP gel is cloned into a plasmid vector and sequenced. The sequence is then used to

design targeted primers for PCR. STAR products are analyzed on a second SSCP gel, and usually segregate as codominant markers.

SSCP analysis of cDNA sequences provides another class of highly polymorphic markers for use in linkage mapping. Although these are unique, functional regions of a genome, polymorphisms are frequent, and can be used to generate linkage maps. This method has been used to map cDNA makers in *A. aegypti* (Fulton *et al.*, 2001).

In this paper we describe the use of MS, RAPD-PCR, STAR, and cDNA markers to generate a preliminary linkage map in an F₁ intercross family of *I. scapularis*. We describe our successes and failures in developing polymorphic markers and provide a preliminary 616 cM linkage map across 14 linkage groups, the 1N number of chromosomes determined cytogenetically (Oliver, 1977). This provided a linkage map with one marker every 10.8 cM. However, counting markers that did not map to any of these 14 linkage groups, we estimate a much larger map size of 3165 cM with an average resolution of only one marker every 55 cM.

Materials and Methods

Mapping cross

All ticks were kept in Wheaton 8 ml sample vials (Wheaton, Millville, NJ) which were placed into glass desiccator jars with water in the bottom to maintain a high relative humidity. The desiccators were maintained in a Revco bioclimatic chamber (Revco Inc., Deerfield, MI) at 21°C, 95% humidity and received a photoperiod of 16:8 (light:dark). ICR outbred, pathogen-free mice were used for blood feedings (DVBID, CDC, Ft. Collins). Four fertilized P₁ females were collected from Bridgeport, Connecticut in the fall of 1997, brought back to DVBID, and fed on rabbits. Eggs were laid by the P₁ females and hatched ~4 weeks later to generate four initial families. After eggs were collected from each P₁ mother, each female was frozen in a labeled cryotube at -20°C in 70% ethanol. F₁ eggs from each female were hatched out and larvae were blood fed on mice. One hundred replete larvae were placed in Wheaton vials and ~5 weeks after being fed, the larvae molted to nymphs. Once the cuticle hardened, the F₁ nymphs were blood fed. At this point the nymphs went into diapause for ~8 months due to an unintentional drop in incubator temperature to 16°C. When the incubator temperature was corrected, the ticks came out of diapause and development continued. When the fed nymphs molted to adults, eight single F₁ intercrosses were set up per family. Successful breeding pairs were established from P₁

female #2 (five pairs), and only a single pair from P₁ females #1, #3, and #4. For each F₁ intercross, females were isolated from the males, and single breeding pairs were made and subsequently fed individually in an ear bag on a rabbit ear.

Fed females were separated into labeled vials to collect F₂ eggs. These were laid from 4 to 6 weeks post-feeding. Each F₁ male was frozen in a labeled tube at -20°C in 70% ethanol, and given the same number as his F₁ mate. Each F₁ female was frozen after collecting eggs. F₂ eggs were hatched and when the cuticle had hardened, blood fed on mice. After the larvae had molted to nymphs, ~ 4 weeks post-feed, and the cuticle had hardened, nymphs were fed on mice. The nymphs were reared to adults, and individually assigned a number that corresponded to the P₁ and F₁ female numbers. The F₂ adults were then frozen in a labeled tube at -20°C in 70% ethanol.

Markers

Microsatellite loci were derived as described by Fagerberg *et al.* (2001). RAPD loci were amplified and run on SSCP gels as described in Black and DuTeau (1997). STAR loci were derived from polymorphic RAPD loci following exactly the procedure in Bosio *et al.* (2000). Primers for cDNA sequences from an *I. scapularis* salivary gland genomic library (kindly provided by Jose Ribeiro) were developed following the methodology of Fulton *et al.* (2001).

Map data analysis

Map distances were converted from recombination fractions to map units (cM) using the Kosambi mapping function (Kosambi, 1944). Offspring genotypes of F₁ and F₂ *I. scapularis* were entered into JoinMap[®] 2.0 (Stam and van Ooijen, 1995). Because the numbers of parental, recombinant, and uninformative genotypes, and the segregation ratios among the F₂ offspring may differ for each locus, JoinMap allows each marker to be analyzed according to parental genotypes. Initially, a threshold recombination fraction of 0.499 and a log odds density (LOD) score of 3.0 was used to group markers. The minimal LOD was then increased to 6.0 in increments of 0.1 to monitor the rate at which markers left individual linkage groups. Increasing the minimum LOD in increments tests how robust are the linkage groups in the genotype data set. DrawMap[®] (van Ooijen, 1994) was used to plot a linkage map from the recombination frequencies generated by JoinMap.

Results

The P₁ generation began as a field-collected, fertilized female. The genotype of the P₁ male had therefore to be inferred at all loci. Recombination frequencies among all marker loci were analyzed in 232 F₂ progeny.

Twenty RAPD primers (Table 1) amplified 84 polymorphic marker loci. Genotypes at 64 loci were in expected Mendelian ratios among the F₂ offspring. Sixty-three of these loci and D04.800a and b (bands ~1 mm apart on the SSCP gel that did not completely cosegregate) were extracted from SSCP gels, reamplified, cloned and sequenced to generate 65 primer pairs (Table 2). Of these, 52 STAR loci were polymorphic, and genotypes at 33 of these were in expected Mendelian ratios in the F₂ offspring. Alleles at 20 of the 33 STAR loci segregated as codominant markers.

Fifty-six sequences from an *I. scapularis* salivary gland cDNA library (sequences kindly provided by Jose Ribeiro) were analyzed for primer design.

Table 1. List of RAPD primers and sequences utilized for mapping

| Primer | Sequence |
|--------|------------|
| A09 | GGGTAACGCC |
| A20 | GTTGCGATCC |
| B15 | GGAGGGTGT |
| B18 | CCACAGCAGT |
| B20 | GGACCCCTAC |
| C19 | GTTGCCAGCC |
| C01 | TTCGAGCCAG |
| C04 | CCGCATCTAC |
| D02 | GGACCCAACC |
| D03 | GTCGCCGTCA |
| D04 | TCTGGTGAGG |
| D07 | TTGGCACGGG |
| D08 | GTGTGCCCCA |
| D12 | CACCGTATCC |
| D13 | GGGGTGAGCA |
| D16 | AGGGCGTAAG |
| D17 | TTTCCCACGG |
| D18 | GAGAGCCAAC |
| D19 | CTGGGGACTT |
| D20 | ACCCGGTCAC |

Table 2. List of 95 primer pairs for PCR amplification of 65 STAR, 20 cDNA and 10 microsatellite loci in *I. scapularis*

| Locus name | | T _a | Length (bp) | Primer sequences |
|------------|------------------------|----------------|-------------|---|
| STARs | | | | |
| A09.306ST | Genbank Acc. #BZ385505 | 49 | 306 | GGGTAACGCCAGGGTTTTCC GGGTAACGCCCGATGTATAG |
| A20.310ST | Genbank Acc. #BZ592381 | 51 | 310 | GTTGCGATCCCTCAGAGCGA GTTGCGATCCAACGAAGTTT |
| A20.390ST | Genbank Acc. #BZ592382 | 57 | 390 | GTTGCGATCCCAGGATATAC GTTGCGATCCTASAGCACAT |
| A20.517ST | Genbank Acc. #BZ592383 | 59 | 517 | GTTGCGATCCCTGCGCCTAT GTTGCGATCCAGGCGATCAC |
| B15.874ST | Genbank Acc. #BZ385506 | 59 | 874 | GGAGGGTGTTCACAGTACA GGAGGGTGTGGGGTGTGTG |
| B18.358ST | Genbank Acc. #BZ385507 | 62 | 358 | CCACAGCAGTCAAACCTTCT CCACAGCAGTAGTGATACTC |
| B18.653ST | Genbank Acc. #BZ385508 | 62 | 653 | CCACAGCAGTCGACCATGCG CCACAGCAGTAGTGATACTC |
| B20.361ST | Genbank Acc. #BZ385509 | 62 | 361 | GGACCCTTACGAGCGAAAGA GGACCCTTACCCCAACCAAA |
| C13.357ST | Genbank Acc. #BZ385510 | 62 | 357 | AAGCCTCGTCGGGGTAGAGA AAGCCTCGTCCTGCTGTTCT |
| C13.579ST | Genbank Acc. #BZ385511 | 60 | 579 | AAGCCTCGTCGTTAGACGTT AAGCCTCGTCCACTTTCCCTT |
| C16.362ST | Genbank Acc. #BZ385512 | 62 | 362 | CACACTCCAGTCAGCATCAG CACACTCCAGGACCAATAGT |

| | | | | |
|-----------|------------------------|----|-----|---|
| C16.432ST | Genbank Acc. #BZ385513 | 46 | 432 | CACACTCCAGGCAAAAAAAC CACACTCCAGCATTAGGGAA |
| C16.458ST | Genbank Acc. #BZ385514 | 59 | 458 | CACACTCCAGGCAGATGCAT CACACTCCAGCATCAAGTGC |
| C16.680ST | Genbank Acc. #BZ385515 | 48 | 680 | CACACTCCACCAATCCCCTA CACACTCCACGATACAAACG |
| C01.169ST | Genbank Acc. #BZ385516 | 59 | 169 | TTCGAGCCAGGGCAGGACCT TTCGAGCCAGCATTGATAAG |
| C01.170ST | Genbank Acc. #BZ385517 | 59 | 170 | TTCGAGCCAGCATTGAGAAG TTCGAGCCAGGGCAGGACCT |
| C04.323ST | Genbank Acc. #BZ385518 | 60 | 323 | CCGCATCTACGTACGATTGA CCGCATCTACGCCCGGTAGT |
| C04.331ST | Genbank Acc. #BZ385519 | 57 | 331 | CCGCATCTACATATACAATA CCGCATCTACCCCATCGACG |
| C04.345ST | Genbank Acc. #BZ385520 | 57 | 345 | CCGCATCTACGTTAGCAGTC CCGCATCTACACACAGTTTC |
| D02.328ST | Genbank Acc. #BZ385392 | 50 | 328 | GGACCCAACCCTATACCGCT GGACCCAACCCTACGGAGGAG |
| D02.330ST | Genbank Acc. #BZ385393 | 48 | 330 | GGACCCAACCCTATACCGCT GGACCCAACCCTACGGAGGAG |
| D02.460ST | Genbank Acc. #BZ385394 | 45 | 460 | GGACCCAACCATGTTTTAAA GGACCCAACCCCAAGAAATC |
| D02.463ST | Genbank Acc. #BZ385395 | 48 | 463 | GGACCCAACCCCAAGAAATC GGACCCAACCATGTTTTAAC |

Table 2. (continued)

| Locus name | | T _a | Length (bp) | Primer sequences |
|------------|-----------------------------------|----------------|-------------|--|
| D02.464ST | Genbank Acc. #BZ385396 | 48 | 464 | GGACCCAACCCCAAGAATCC GGACCCAACCATGTTTTAAC |
| D02.466ST | Genbank Acc. #BZ385397 | 48 | 466 | GGACCCAACCCCAAGAAATC GGACCCAACCATGTTTTAAC |
| D02.470ST | Genbank Acc. #BZ385398 | 50 | 470 | GGACCCAACCATGTTTTAAC GGACCCAACCCCAAGAATC |
| D02.472ST | Genbank Acc. #BZ385399 | 48 | 472 | GGACCCAACCATGTTTTAAA GGACCCAACCCCAAGAATCC |
| D04.410ST | Genbank Acc. #BZ385400 | 45 | 410 | TCTGGTGAGGGGAAGAAGG TCTGGTGAGGCAACTACTGA |
| D04.534ST | Genbank Acc. #BZ385401 | 45 | 534 | TCTGGTGAGGCGTTCAAGGC TCTGGTGAGGAGGAATGTTC |
| D04.800AST | Genbank Acc. #BZ85433- BZ85434 | 45 | 800 | TCTGGTGAGGTTCGTGTCAA TCTGGTGAGGCGGGGCTCCA |
| D04.800BST | Genbank Acc. #BZ85435- BZ85436 | 45 | 800 | TCTGGTGAGGCGGGACTTCA TCTGGTGAGGTTCGTGTCAA |
| D07.292ST | Genbank Acc. #BZ385402 | 45 | 292 | TTGGCACGGGCACACAAATA TTGGCACGGGGCTGCAACAA |
| D07.434ST | Genbank Acc. #BZ385403 | 50 | 434 | TTGGCACGGGCAATCATTGC TTGGCACGGGACGCGGAAGC |

| | | | | |
|------------|------------------------------------|----|------|--|
| D07.457ST | Genbank Acc. #BZ385404 | 50 | 457 | TTGGCACGGGCGCGTGGCTT TTGGCACGGGGGACAAAAGA |
| D07.649ST | Genbank Acc. #BZ385405 | 50 | 649 | TTGGCACGGGCAGCAGCCGG TTGGCACGGGTGGAGTTCGT |
| D08.459ST | Genbank Acc. #BZ385406 | 50 | 459 | GTGTGCCCCAGGACCTGGGT GTGTGCCCCACCGCAAAGAG |
| D08.463ST | Genbank Acc. #BZ385407 | 50 | 463 | GTGTGCCCCACCGCAAAGAG GTGTGCCCCAGGACCTGGGT |
| D12.425ST | Genbank Acc. #BZ385408 | 50 | 425 | CACCGTATCCAGGAAGTGTT CACCGTATCCTCGCAGAGTT |
| D12.617ST | Genbank Acc. #BZ385409 | 36 | 617 | CACCGTATCCCCCTAGCGAA CACCGTATCCAACAAAGGAT |
| D12.821ST | Genbank Acc. #BZ385410 | 43 | 821 | CACCGTATCCCGTTGGCCTA CACCGTATCCCCATGATTTC |
| D12.942ST | Genbank Acc. #BZ385411 | 50 | 942 | CACCGTATCCATGTGTAATC CACCGTATCCCCAGACTCGG |
| D17.459ST | Genbank Acc. #BZ385412 | 45 | 459 | TTTCCCACGGGCACGTACTA TTTCCCACGGCGTCATGACT |
| D17.984ST | Genbank Acc. #BZ385413 | 48 | 984 | TTTCCCACGGCAGGATAACT TTTCCCACGGATTAATCAAA |
| D17.1200ST | Genbank Acc. #BZ385437- BZ85438 | 43 | 1200 | TTTCCCACGGAACGACTCAC TTTCCCACGGCCAAACAAAG |
| D13.363ST | Genbank Acc. #BZ385414 | 50 | 363 | GGGGTGACGACCGACTGGC GGGGTGACGAGACAACGAAA |

Table 2. (continued)

| Locus name | | T _a | Length (bp) | Primer sequences |
|------------|------------------------|----------------|-------------|--|
| D13.443ST | Genbank Acc. #BZ385415 | 50 | 443 | GGGGTGACGATGGGTTGTGG GGGGTGACGACCAACCCGGG |
| D16.330ST | Genbank Acc. #BZ385416 | 50 | 330 | AGGGCGTAAGCAGAACCGTT AGGGCGTAAGCATCAGGTAT |
| D16.470ST | Genbank Acc. #BZ385417 | 50 | 470 | AGGGCGTAAGGGCCGATAGC AGGGCGTAAGTGCCTGTGTG |
| D16.521ST | Genbank Acc. #BZ385418 | 50 | 521 | AGGGCGTAAGCCTAACAAAA AGGGCGTAAGGGTAGTGTGT |
| D17.684ST | Genbank Acc. #BZ385419 | 50 | 684 | TTTCCCACGGCACCCTTTGT TTTCCCACGGGCCATTCCGC |
| D18.265ST | Genbank Acc. #BZ385420 | 41 | 265 | GAGAGCCAACACGTCAAATA GAGAGCCAACCTCACTGACC |
| D18.266ST | Genbank Acc. #BZ385421 | 43 | 266 | GAGAGCCAACCTCACTGACCT GAGAGCCAACACGTCAAAT |
| D18.284ST | Genbank Acc. #BZ385422 | 39 | 284 | GAGAGCCAACCTACTTGGCA GAGAGCCAACATGGAGAGGA |
| D18.353ST | Genbank Acc. #BZ385423 | 39 | 353 | GAGAGCCAACGGTAAATAG GAGAGCCAACAAGAGACTAC |
| D18.366ST | Genbank Acc. #BZ385424 | 50 | 366 | GAGAGCCAACCCACCTTCAT GAGAGCCAACGCGGGGCGA |
| D18.367ST | Genbank Acc. #BZ385425 | 50 | 367 | GAGAGCCAACGCGGGGCGA GAGAGCCAACCCACCTACAT |

| | | | | |
|--|------------------------|----|-----|--|
| D18.369ST | Genbank Acc. #BZ385426 | 50 | 369 | GAGAGCCAACCCACCTTCAT GAGAGCCAACCCGCGGGCGA |
| D18.826ST | Genbank Acc. #BZ385427 | 50 | 826 | GAGAGCCAACAATCAACCTC GAGAGCCAACGCCAAAGAAA |
| D19.382ST | Genbank Acc. #BZ385428 | 50 | 382 | CTGGGGACTTCAAACAACCC CTGGGGACTTGCCACGTCAG |
| D19.833ST | Genbank Acc. #BZ385429 | 50 | 833 | CTGGGGACTTCACAGAGGCA CTGGGGACTTCCGTTTGGAT |
| D20.284ST | Genbank Acc. #BZ385430 | 50 | 284 | ACCCGGTCACCAATTTGTAG ACCCGGTCACAGATGGAGAA |
| D20.294ST | Genbank Acc. #BZ385431 | 50 | 294 | ACCCGGTCACACGAGCGCTT ACCCGGTCACGTCTTCACTG |
| D20.817ST | Genbank Acc. #BZ385432 | 50 | 817 | ACCCGGTCACCAGCAGATTT ACCCGGTCACATCATTGTGT |
| cDNA ^a | | | | |
| Is218 (APOP-IN (AF333765) DAD-1-like protein [<i>Schistosoma japonicum</i>] (3.00E-41)*) | Genbank Acc. #CA763761 | 60 | 209 | GCCAGCGTAAACGAAACC GATGAAGCCCCGACAGGAA |
| Is155 (ATP-S (mitochondrial ATP synthase [<i>Drosophila melanogaster</i>] (1.00E-25)) | Genbank Acc. #CA763762 | 52 | 195 | CTGAAAGGGTCCCAGAAGA GGAATGGCACCGTAAAGC |

Table 2. (continued)

| Locus name | | T _a | Length (bp) | Primer sequences |
|---|------------------------|----------------|-------------|--|
| Is108 (EF1-A (AF378368 elongation factor 1-alpha [<i>Coccidioides</i> <i>immitis</i>] (3.00E-34)) | Genbank Acc. #CA763763 | 62 | 196 | TGTGGGCGTCATCAAGTC AAACAGAGTAACCAAACGAAAG |
| Is119 (ELF-2B (CAC08449 eukaryote initiation factor 2 beta [<i>Gallus gallus</i>] (5.00E-57)) | Genbank Acc. #CA763764 | 58 | 301 | CCTTCGCCAACTTCCTCG AAGCCAGACTTGATGCTCAC |
| Is211 (GST-1 (AF366931 glutathione S-transferase [<i>Boophilus</i> <i>microplus</i>] (1.00E-30)) | Genbank Acc. #CA763765 | 53 | 218 | GAAATAACGGTTGAGGGC GTAGGGAAGGTTGGGAAA |
| Is75 (GST-2 (JX0095 glutathione transferase (EC 2.5.1.18) b – guinea pig) (1.00E-30)) | Genbank Acc. #CA763766 | 53 | 164 | TCCTCGTTGAGATCCAGT GTTCCAGCAGTAATAGTCG |
| ISAC (AF270496 <i>I.</i> <i>scapularis</i> anticomplement protein) | | 56 | 188 | TTTTGGCGATTTCGTTTC AATTCCTTCCCAGGTTGC |
| Is152 (ND(CG7580 gene product [<i>D.</i> <i>melanogaster</i>] putative NADH dehydrogenase) (6.00E-27)) | Genbank Acc. #CA763767 | 53 | 251 | GCACTTTGGGAACCTGAT TGGGCTGCTTCTACTTGTC |

| | | | | |
|--|------------------------|----|-----|--|
| Is136 (QM (XP 141003 similar to ribosomal protein L10 [<i>Rattus norvegicus</i>] (2.00E-49)) | Genbank Acc. #CA763768 | 62 | 184 | AGGCCAAGGTGGATGAGTT TTGTTGATGCCGATGACG |
| Is30 (RPS12 (AF470687 40S ribosomal protein S12 [<i>Branchiostoma belcheri</i>] (1.00E-49)) | Genbank Acc. #CA763769 | 62 | 214 | GTCATCTGTGCGTCCTG TCTCCTTGCCGTAGTCCTT |
| Is140 (RPS13 (AF116857 40S ribosomal protein S13 [<i>Cricetulus griseus</i>] (3.00E-48)) | Genbank Acc. #CA763770 | 60 | 177 | CCCAGTCGGCTCTGCCATAC TCTTGTTGCCCGTCACCC |
| Is45 (RPS14B (AF402822 40S ribosomal protein S14 [<i>Ictalurus punctatus</i>] (3.00E-52)) | Genbank Acc. #CA763771 | 53 | 183 | TTTGGCGTAGCCCACATA GACCTGCTTGCATTTCTCG |
| Is10 (RPS18) (AAN52390 ribosomal protein S18 [<i>B. belcheri</i>] (1.00E-65)) | Genbank Acc. #CA763772 | 55 | 206 | TCGCCCTGACAGCCATTA CTGAACTTGCCGTCCTTG |
| Is66 (RPL12 (AAH08230 ribosomal protein L12 [<i>Homo sapiens</i>] (1.00E-49)) | Genbank Acc. #CA763773 | 61 | 310 | CCCAAGTTTGTATCCCACG ATGTCAGGTTGCCGCTGT |

Table 2. (continued)

| Locus name | | T _a | Length (bp) | Primer sequences |
|---|------------------------|----------------|-------------|--|
| Is149 (RPL17A) (AF395586 ribosomal protein L17/23 [<i>Spodoptera frugiperda</i>] (1.00E–57)) | Genbank Acc. #CA763774 | 44 | 293 | GCATCTCCCTCGGTCTTC ATTCGCCTTTGTTGTTGA |
| Is171 (RPL40) Ribosomal Protein L40 ubiquitin extension protein [<i>D. melanogaster</i>] (2.00E–41)) | Genbank Acc. #CA763775 | 68 | 154 | GACTGGTGGGTGGTGTTCATT GGTCGCAGGTTGTTGGAG |
| Is28 (RPL44) (AAM94276 ribosomal protein L44 [<i>Chlamys farreri</i>]) (3.00E–49)) | Genbank Acc. #CA763776 | 45 | 250 | ATGATAAGAAGGGCAAGGAT CTGGTGGATTAGAACTGGAT |
| Is27 (PRP (AF400199 ribosomal protein L37 [<i>S. frugiperda</i>] (6.00E–30)) | Genbank Acc. #CA763777 | 58 | 152 | GCAGCTACCACATCCAAA CTGAAACGCCTCCAGACC |
| SALP16 (AF061845 Ixodes scapularis salivary gland 16 kDa protein (salp16)) | | 63 | 371 | CAGTGAGACGGGAGCATC GTTCGGCACCTTCCTTTA |
| Is79 (UBQ (CAA44453 ubiquitin-conjugating enzyme [<i>D. melanogaster</i>] (2.00E–43)) | Genbank Acc. #CA763778 | 47 | 196 | ACAGCAATGGCAGCATCT TTCCTGGTCCACTCCCTA |

| Microsatellites | | | | |
|-----------------|---------------------------------|----|-----|--|
| ISAG4 | Genbank Acc. #AF331743 | 41 | 250 | AAGAAAATAAAGCGAACAAG ATAAGCAATTCATACGAGATAGT |
| ISAG25 | Genbank Acc. #AF331742 | 57 | 171 | AAATGTCCGAACAGCCTTAT GCCCTTGAGTCTACCCACTA |
| ISAC4 | Genbank Acc. #AF331736-AF331739 | 49 | 170 | GGGTCCCAACGATTGCTAAACCAG AAGCGTATCCGATTTGCCCTTCAT |
| ISAC8 | Genbank Acc. #AF331740 | 42 | 170 | TCTTCCCCTGCTGTCTCGTATTC GAGCTACCCCTTTCATCGTCTTCG |
| ISAC20 | Genbank Acc. #AF331734 | 42 | 150 | AGAAACACGGAAGGAGAAAGGAGA AACTGTGCCAGATGGGAAAGAAGA |
| ISAC22 | Genbank Acc. #AF331735 | 44 | 200 | CAGCTGGGCCCTCCTTTTAATCC TATTGTAAGGCCAGTCGCCGCTGC |
| ISCAG12 | Genbank Acc. #AF331755 | 44 | 170 | GAAGAACACCGAGCGAACCAGAAC GTGAGCTGAGGGTTGCTGTTGATG |
| ISGATA3 | Genbank Acc. #AF331752 | 47 | 150 | AGTCCCCTCAGAGCGATTTTCA GGCCGCCAGTTTGATGGATA |
| ISGATA4 | Genbank Acc. #AF331753 | 41 | 200 | CAGACAATGTCATTCAATCGCA CGCACAATGCAAAACAATCTA |
| ISCTGY17 | Genbank Acc. #AF331745-AF331747 | 41 | 150 | TTCTTGTTTTATTGGTTGGGTG AATGCAGGGTAAGTTGAGATTG |

^a * designates E score for cDNAs of unknown function. Information following the IS# designations on cDNAs indicate the results of a BLASTX search on Genbank.

Primers were designed to amplify 250–400 bp fragments (optimal size for SSCP) in 20 of the genes (Table 2) with high matches to existing genes. However, ultimately only five of these could be mapped because the genomic DNA amplified by the primers was much larger than the anticipated size based upon the cDNA sequence. Amplified products from these loci were extracted from the polyacrylamide gels, reamplified and cloned into a plasmid vector for subsequent sequencing. As expected we found that the cDNA sequence was interrupted by large introns, some up to 800 bp in size. SSCP analysis can only reliably reveal polymorphisms in fragments <500 bp. We did not design internal primers from the genomic sequences.

Primers for 10 MS loci (Fagerberg *et al.*, 2001) are also listed in Table 2. However, only six of these could be mapped, one of which was not in expected Mendelian ratios, consequently only five MS markers have been mapped. The remaining four were monomorphic, further supporting the observations of low heterozygosity found at *I. scapularis* MS loci (Fagerberg *et al.*, 2001).

A linkage map was generated using JoinMap (Figure 1) (Ullmann *et al.*, 2003). The linkage map contains only those loci at which genotypes were in Mendelian ratios. Fourteen linkage groups were identified by JMGRP at an LOD of 3.0. Above this LOD, markers began splitting away either singly or into separate groups. A total of 57 markers mapped across 616 cM on the 14 linkage groups with one marker every 10.8 cM. A total map distance of 3166 cM was estimated by making the assumption that the remaining 51 markers that did not cosegregate onto any of the 14 linkage groups were ≥ 50 cM from mapped markers ($51 \text{ markers} \times 50 \text{ cM/marker} + 616 \text{ cM} = 3166 \text{ cM}$).

Linkage groups on the map are temporarily assigned numbers according to their relative lengths. These lengths could change if more markers are added to the map. Ultimately numbers will be assigned to chromosomes *via* physical mapping when a physical map for *I. scapularis* exists. When this is accomplished then chromosome numbers will be assigned according to overall chromosome length. Currently, the map shows that linkage group 1 is four times longer than any of the other 13 linkage groups. We suspect that linkage group 1 corresponds to the sex chromosome, because it is known from cytogenetic work that in *Ixodes* the sex chromosome is three to four times longer than the autosomes (Oliver, 1977), and when sex was mapped it was most closely linked to A09.583 on linkage group 1 with an LOD of 2.667.

The size of this linkage maps suggests a very high recombination rate for the *I. scapularis* genome. The physical size of another ixodid tick genome, *Amblyomma americanum*, was previously determined to be 1.08 pg or 1.04×10^9 bp, and repetitive elements were in a long-period interspersed pattern (Palmer *et al.*, 1994). Assuming this is an accurate estimate of the size of the

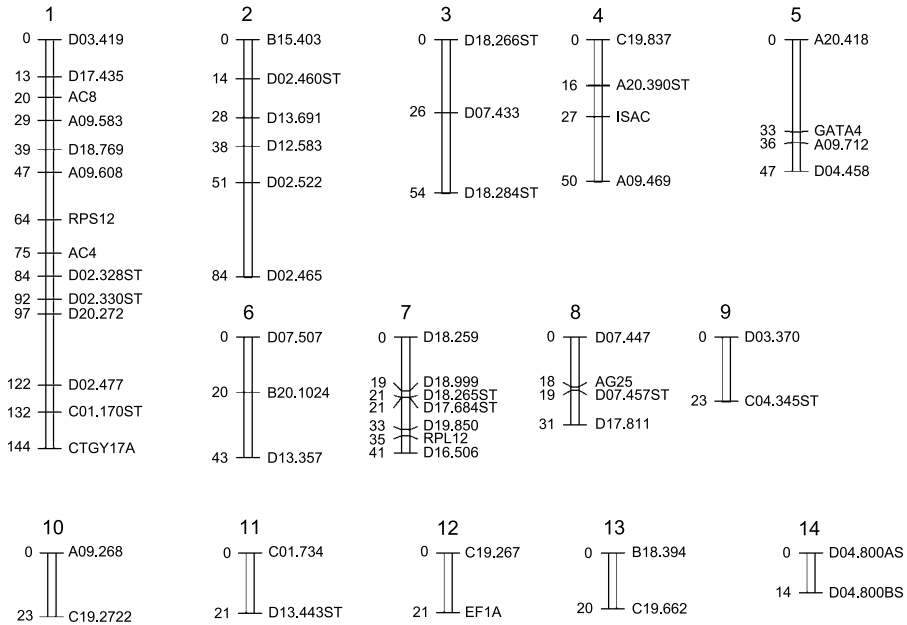


Figure 1. A preliminary linkage map of the *I. scapularis* genome that includes only those loci at which genotypes were in Mendelian ratios. Fourteen linkage groups were identified by JMGRP at an LOD of 3.0. A total of 57 markers map across 616 cM on 14 linkage groups with one marker every 10.8 cM. Linkage groups on the two maps are temporarily assigned numbers according to their relative linkage distances. These do not necessarily correspond to chromosomes numbered according to length cytogenetically (Oliver, 1977). RAPD markers are indicated by the name of the Operon primer, a period followed by the size of the amplified fragment. A STAR locus is indicated by a RAPD label followed by an 'ST'. cDNA loci follow the labels designated in Valenzuela *et al.* (2002). Microsatellite loci are indicated by AC, AG, CTGY, or GATA and the clone number.

I. scapularis genome, then the relationship of physical to genetic distance is $\sim 267\text{--}329$ kb/cM. Work is currently underway to determine the physical size and organization of the *I. scapularis* genome.

Discussion

Our experience in generating this preliminary linkage map demonstrates both promising aspects to and pitfalls for future work in tick genetics and genomics. Having tried conventional RAPDs, STARS, MS, and cDNAs, it appears that STARS are likely to be the marker of choice for expanding the *I. scapularis* linkage map and eventually integrating the linkage map into a physical map. Of the 65 STAR primer sets designed (Table 2), 52 were polymorphic and genotypes at 37 of these conformed to expected Mendelian ratios. The

numbers of RAPD loci and the amount of heterozygosity at these loci were not as great as those detected in *A. aegypti* (Antolin *et al.*, 1996). From the 20 RAPD primers, a total of 63 markers were mapped to the *I. scapularis* genome while 94 markers were generated with only 10 RAPD primers in an *Ae. aegypti* F₁ intercross family (Antolin *et al.*, 1996). Reassociation kinetic studies of another ixodid tick, *A. americanum*, showed that the organization of repetitive DNA was of the long-period interspersion type (Palmer *et al.*, 1994). We suspect that this is also the case for *I. scapularis*.

Fagerberg *et al.* (2001) had to use a specialized capture technique to identify a few MS loci in *I. scapularis*. Furthermore, the heterozygosity at these few loci was low. We have no explanation for the low abundance and variability at MS loci in *I. scapularis*. Sequences obtained from cDNA libraries were useful in generating PCR primers that amplified single nucleotide polymorphisms (SNPs) for linkage mapping in an *A. aegypti* F₁ intercross family (Fulton *et al.*, 2001). In most cases the amplified regions were of the same size as the cDNA. However, this was not the case with cDNAs in the *I. scapularis* genome. In only 5 of the 20 cDNAs, for which primers were designed, were we able to recover a fragment that was sufficiently short for SSCP analysis. This appears to be due to the presence of large intervening introns. Taking the additional steps required to identify the locations and sequences of intron/exon boundaries will be very laborious and expensive.

We are in the process of performing the reassociation kinetics of the *I. scapularis* genome. However, assuming that the physical size of the *I. scapularis* genome is approximately the same size as the *A. americanum* genome (1.04×10^9 bp) (Palmer *et al.*, 1994), perhaps an inappropriate assumption to make, our results suggest that there is a great deal of recombination in *I. scapularis*. A resolution of ~ 300 kb/cM compares favorably to the average 1100 kb/cM resolution in *A. gambiae* (Dimopoulos *et al.*, 1996) or the 1000–3400 kb resolution for *A. aegypti* (Brown *et al.*, 2001). A map of this resolution has a higher probability of being successfully used in mapped-based positional cloning of candidate genes for vector competence to pathogens, host preference, insecticide resistance and other important characters in *I. scapularis*.

References

- Antolin, M.F., Bosio, C.F., Cotton, J., Sweeney, W., Strand, M.R. and Black, W.C.t. 1996. Intensive linkage mapping in a wasp (*Bracon hebetor*) and a mosquito (*Aedes aegypti*) with single-strand conformation polymorphism analysis of random amplified polymorphic DNA markers. *Genetics* 143: 1727–1738.
- Black, W.C. and DuTeau, N.M. 1997. In: *The Molecular Biology of Insect Disease Vectors: A Methods Manual*, pp. 361–373. Chapman & Hall, New York.

- Bosio, C.F., Fulton, R.E., Salasek, M.L., Beaty, B.J. and Black, W.C.t. 2000. Quantitative trait loci that control vector competence for dengue-2 virus in the mosquito *Aedes aegypti*. *Genetics* 156: 687–698.
- Brown, S.E., Severson, D.W., Smith, L.A. and Knudson, D.L. 2001. Integration of the *Aedes aegypti* mosquito genetic linkage and physical maps. *Genetics* 157: 1299–1305.
- Dietrich, W., Katz, H., Lincoln, S.E., Shin, H.S., Friedman, J., Dracopoli, N.C. and Lander, E.S. 1992. A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131: 423–447.
- Dimopoulos, G., Zheng, L., Kumar, V., della Torre, A., Kafatos, F.C. and Louis, C. 1996. Integrated genetic map of *Anopheles gambiae*: use of RAPD polymorphisms for genetic, cytogenetic and STS landmarks. *Genetics* 143: 953–960.
- Fagerberg, A.J., Fulton, R.E. and Black, W.C. 2001. Microsatellite loci are not abundant in all arthropod genomes: analyses in the hard tick, *Ixodes scapularis* and the yellow fever mosquito, *Aedes aegypti*. *Insect Mol. Biol.* 10: 225–236.
- Fulton, R.E., Salasek, M.L., DuTeau, N.M. and Black, W.C.t. 2001. SSCP analysis of cDNA markers provides a dense linkage map of the *Aedes aegypti* genome. *Genetics* 158: 715–726.
- Hayashi, K. 1991. PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA. *PCR Meth. Appl.* 1: 34–38.
- Hiss, R.H., Norris, D.E., Dietrich, C.H., Whitcomb, R.F., West, D.F., Bosio, C.F., Kambhampati, S., Piesman, J., Antolin, M.F. and Black, W.C.t. 1994. Molecular taxonomy using single-strand conformation polymorphism (SSCP) analysis of mitochondrial ribosomal DNA genes. *Insect Mol. Biol.* 3: 171–182.
- Hudson, T.J., Stein, L.D., Gerety, S.S., Ma, J., Castle, A.B., Silva, J., Slonim, D.K., Baptista, R., Kruglyak, L., Xu, S.H. et al. 1995. An STS-based map of the human genome. *Science* 270: 1945–1954.
- Hunt, G.J. and Page Jr., R.E. 1995. Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. *Genetics* 139: 1371–1382.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12: 172–175.
- Oliver Jr., J.H. 1977. Cytogenetics of mites and ticks. *Annu. Rev. Entomol.* 22: 407–429.
- Orita, M., Suzuki, Y., Sekiya, T. and Hayashi, K. 1989. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 5: 874–879.
- Palmer, M.J., Bantle, J.A., Guo, X. and Fargo, W.S. 1994. Genome size and organization in the ixodid tick *Amblyomma americanum* (L.). *Insect Mol. Biol.* 3: 57–62.
- Sonenshine, D.E. 1991. *Biology of Ticks*, Vol. 1, New York.
- Spielman, A. 1976. Human babesiosis on Nantucket Island: transmission by nymphal *Ixodes* ticks. *Am. J. Trop. Med. Hyg.* 25: 784–787.
- Spielman, A., Wilson, M.L., Levine, J.F. and Piesman, J. 1985. Ecology of *Ixodes dammini*-borne human babesiosis and Lyme disease. *Annu. Rev. Entomol.* 30: 439–460.
- Stam, P. and van Ooijen, J.W. 1995. CPRO-DLO, Wageningen, The Netherlands.
- Telford III, S.R., Dawson, J.E., Katavolos, P., Warner, C.K., Kolbert, C.P. and Persing, D.H. 1996. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proc. Natl. Acad. Sci. USA* 93: 6209–6214.
- Ullmann, A.J., Piesman, J., Dolan, M.C. and Iv, W.C. 2003. A preliminary linkage map of the hard tick, *Ixodes scapularis*. *Insect Mol. Biol.* 12: 201–210.
- van Ooijen, J.W. 1994. DrawMap: a computer program for drawing genetic linkage maps. *J. Hered.* 85: 66.

- Vidal-Puig, A. and Moller, D.E. 1994. Comparative sensitivity of alternative single-strand conformation polymorphism (SSCP) methods. *Biotechniques* 17: 490–492, 494, 496.
- Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531–6535.
- Yasukochi, Y. 1998. A dense genetic map of the silkworm, *Bombyx mori*, covering all chromosomes based on 1018 molecular markers. *Genetics* 150: 1513–1525.
- Zheng, L., Benedict, M.Q., Cornel, A.J., Collins, F.H. and Kafatos, F.C. 1996. An integrated genetic map of the African human malaria vector mosquito, *Anopheles gambiae*. *Genetics* 143: 941–952.