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Tick [Genome Mapping]

Amy J. Ullmann
Centers for Disease Control and Prevention, Fort Collins, CO

Jeffrey J. Stuart
Purdue University, stuartjj@purdue.edu

Catherine A. Hill
Purdue University

8 Tick

Amy J. Ullmann[1], Jeffrey J. Stuart[2], and Catherine A. Hill[2]

[1] Division of Vector Borne-Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, CO 80521, USA
[2] Department of Entomology, Purdue University, 901 West State Street, West Lafayette, IN 47907, USA
e-mail: hillca@purdue.edu

8.1 Introduction

Ticks (subphylum Chelicerata: class Arachnida: subclass Acari: superorder Parasitiformes: order Ixodidae) are obligate blood-feeding ectoparasites of global medical and veterinary importance. Ticks live on all continents of the world (Steen et al. 2006). There are approximately 899 species of ticks; the majority are ectoparasites of wildlife and approximately 10% of these are recognized as disease vectors or for their ability to cause direct damage through blood feeding (Jongejan and Uilenberg 2004). Ticks transmit a greater variety of viruses, bacteria, and protozoa than any other blood-feeding arthropod (Dennis and Piesman 2005) and are second only to mosquitoes in terms of their medical and veterinary impact (Sonenshine 1991). Other forms of injury attributed to ticks include anemia, dermatosis, and toxicosis. Worldwide there is growing concern because tick-borne infectious diseases are emerging and resurging (Walker 1998, 2005; Telford and Goethert 2004). Many aspects of tick biology have been investigated at the organismal level. However, efforts to understand the genetic basis of host seeking and selection, attachment and feeding, tick-host-pathogen interactions, development and reproduction, and acaricide resistance have been hindered by a lack of tick nucleotide sequence. This situation is rapidly changing with the recent initiation of large-scale sequencing efforts for several tick species. There has been some effort to develop genetic and physical maps to support and exploit tick genomic data but further advances are urgently required. This chapter provides an overview of the current state of tick genomics and highlights areas for future research.

8.1.1 Phylogeny and Evolution of the Ixodida

Ticks and mites are members of the subclass Acari within the subphylum Chelicerata. The chelicerate lineage is thought to be ancient, having diverged from Trilobites during the Cambrian explosion (Brusca and Brusca 1990). It is estimated that is has been approximately 490–550 million years since arthropods in the subphylum Mandibulata, containing the order Hexapoda (Insects), shared a common ancestor with species in the subphylum Chelicerata (Klompen et al. 1996). Not surprisingly then, ticks differ from other blood-feeding arthropods in many aspects of their biology.

Numerous papers have reviewed the phylogeny, evolution, and historical zoogeography of ticks and mites. Unfortunately, phylogenetic studies of the Acari have been confounded by the lack of fossil evidence, specimens, and molecular data. The current understanding of Ixodida phylogeny is represented in Fig. 1. The suborder Parasitiformes includes the order Ixodida which comprises three families, namely the Argasidae (soft ticks), the Ixodidae (hard ticks), and the Nuttalliellidae (comprising a single species which has not been collected for many years). It is generally accepted that the Ixodidae are divided into two lineages, the Prostriata which consists of the single genus *Ixodes* (subfamily: Ixodinae) containing approximately 249 species, and the Metastriata (all other genera) which contains approximately 464 species. The Prostriata are thought to be a paraphyletic lineage, with one distinct clade comprising Australasian species, and the other clade of non-Australasian species (Klompen et al. 1996). The Metastriata contains four subfamilies, namely Amblyomminae, Bothriocrotoninae, Haemaphysalinae,

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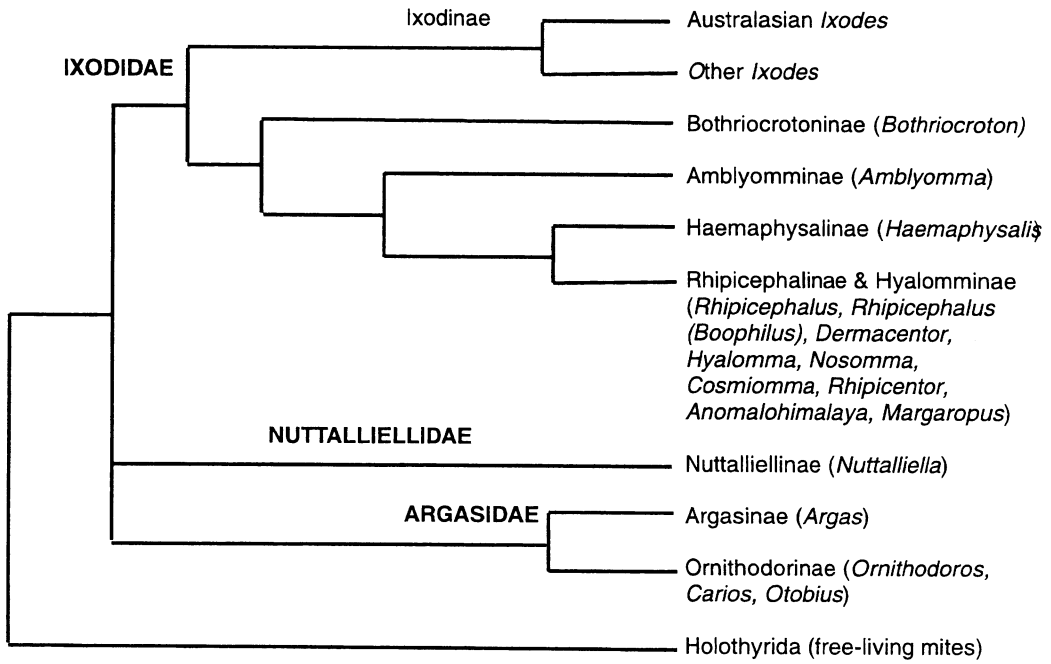

Fig. 1 Current hypothesis of the phylogeny of the subfamilies of ticks. [Reprinted from Toxicon, vol 47, NA Steen, SC Barker, PF Alewood, Proteins in the saliva of the Ixodida (ticks): pharmacological features and biological significance, pp 1–20, Copyright (2006), with permission from Elsevier]

and Rhipicephalinae. The evidence for *Amblyomma* indicates a paraphyletic lineage where *Amblyomma* from Africa form a single lineage and several subgenera of *Amblyomma* exist in South America and Australia. Bothriocrotoninae is a recently added subfamily comprised of the single genus *Bothriocroton*, a basal lineage of endemic Australian ticks previously classified in the genus *Aponomma*. The remaining *Aponomma* species are now considered *Amblyomma* (Klompen et al. 2002). The position of the Haemaphysalinae has yet to be resolved, due in part to incomplete specimens for this genus, as well as morphological similarity to certain *Amblyomma* spp. Hyalomminae (Barker and Murrell 2004) is now considered an invalid subfamily as molecular data suggest that it arises within the Rhipicephalinae (Barker 1998).

Hard ticks are thought to have evolved from bird-feeding soft ticks similar to *Argas* (Black and Piesman 1994; Ribeiro et al. 2006). Klompen et al. (2000) applied a total-evidence based approach utilizing both molecular and morphological characters to propose that the close relationship between holothyrid mites (Acari: Parasitiformes) and the Ixodida suggests that ticks may have evolved from scavengers and not predators. The hypothesis of Steen et al. (2006) suggests that ticks evolved from a saprophytic, carrion-feeding lifestyle, to an obligate hematophagous lifestyle through behavioral, anatomical, and salivary gland adaptations. There has been little molecular analysis of the Argasidae to date although the morphology and systematics of this family have been studied by Klompen (1992) and Klompen and Oliver (1993). Two main hypotheses have been proposed to explain the origin of the hard ticks and their subsequent dispersal around the globe, both of which suggest an origin in that part of Gondwana that eventually became Australia. The first proposes that ticks evolved in Australia on early amphibians in the Devonian ca. 390 Mya (Dobson and Barker 1999) and the other suggests that the first hard tick lived much later (120 Mya) after Australia became relatively isolated (Klompen et al. 1996).

Despite the importance of many *Ixodes* species as parasites and vectors, little is known about the phylogeny of the Ixodinae and there is some debate as to whether the genus *Ixodes* is mono- or para-

phyletic (Barker and Murrell 2002; Xu et al. 2003). Murrell et al. (2001) utilized a total-evidence approach to propose their hypothesis of the origin of Rhipicephalinae. They speculate that the *Dermacentor* lineage evolved in Afrotropical forest and subsequently dispersed on mammals to Eurasia during the Eocene (50 Mya). These ticks subsequently dispersed from Eurasia to the Neartic through the Bering land bridge, and from Europe via Greenland during the Oligocene (35 Mya). The dispersal of the *Dermacentor-Anocentor* ticks from the Neartic to the Neotropics through the Isthmus of Panama took place much later, approximately 2.5 Mya. The *Nosoma-Hyalomma* lineage evolved in the Orient and dispersed during the Miocene (19 Mya). The lineage of *Boophilus* evolved in Africa and dispersed to Eurasia during the Miocene period (14 Mya). Lastly, the *Rhipicentor* species are thought to have evolved in Africa where they have remained confined (Murrell et al. 2001).

8.1.2 Medical, Veterinary, and Economic Importance of Ticks

Some of the most significant tick-borne diseases of humans and animals and their tick vectors are shown in Table 1. The causative agents of these diseases include bacteria (both extracellular and intracellular), viruses, and piroplasm protozoans. The success of ticks as vectors of disease-causing agents can be attributed to a number of factors including wide host range, feeding on multiple hosts, as well as the mechanism and length of time required to blood feed. The long life span (1–2 years) of most hard ticks also enhances vector capability because it provides sufficient time for ticks to become a reservoir host. Both trans-stadial and transovariole mechanisms of pathogen transmission are documented in the Ixodida. Thus, in certain species, both immature stages and adult ticks are competent vectors.

The *Ixodes ricinus* species complex comprises a group of ticks that are distributed in almost all geographic regions of the world and includes a number of species of significance to human health because they vector tick-borne encephalitis virus, rickettsiae, piroplasma, and the *Borrelia* spirochete (Delaye et al. 1997). This complex includes the *Ixodes scapularis* (black-legged or deer tick) and *I. pacificus* (western black-legged tick) vectors of Lyme disease (LD) in the USA, southern Canada, and northern Mexico and the *I. ricinus* and *I. persulcatus* vectors of *Borrelia* in the Palearctic and Oriental regions (Xu et al. 2003). LD is the most common vector borne disease in the USA. Despite federal, state, and local efforts to prevent and control LD, a total of 23,763 cases were reported in 2002 (CDC 2002) representing an almost threefold increase since 1991. The average direct and indirect medical expenses associated with LD patient care are estimated at $2,970 and $5,202 respectively, which translates to a nationwide estimated annual economic impact of approximately US $203 million (in 2002 dollars) (Zhang et al. 2006).

Rhipicephalus (Boophilus) microplus (hereafter *Boophilus*), the tropical or southern cattle tick, has colonized most of the world's tropical and subtropical countries (McCosker 1979; Murrell et al. 2001) and is the most economically important *Boophilus* species. *R. microplus* is a vector of the protozoan (*Babesia bovis* and *B. bigemina*) and bacterial (*Anaplasma marginale*) organisms which cause bovine babesiosis and anaplasmosis ("tick fever"), respectively. The tick-disease complex of *Boophilus* spp.–*Babesia* spp.–*Anaplasma marginale* is probably the most important affecting worldwide livestock production (deCastro 1977), leading to severe economic losses in milk and beef production and restriction in traffic of animals, costing more than US $2.5 billion annually. Chemical treatments (acaricides) are relied on for tick control, however tick resistance to synthetic pyrethroid, organophosphate, and amitraz acaricides is widespread (Foil et al. 2004). Control of cattle ticks is required to minimize production losses and industries incur more than US $200 million in annual losses due to the impact of ticks and tick-borne diseases and costs of treatment to ensure compliance with regulatory protocols for intrastate, interstate, and international livestock movement (Playford and Services 2005).

Other species of ticks that are of medical or veterinary importance include *Rhipicephalus appendiculatus* (brown ear tick) which vectors *Theileria parva*, the causative agent of East Coast fever. In eastern and southern Africa, this disease severely limits cattle production. The tropical bont tick, *Amblyomma variega-*

Table 1 Diseases transmitted by ixodid ticks, showing causative agents and primary tick vector(s)

Disease	Causative agent	Primary tick vectors
Tularemia	*Franciscella tularemia*	*Amblyomma americanum, Dermacentor variabilis, D. nuttalli, Ixodes ricinus*
Human granulocytic ehrlichiosis	*Anaplasma chaffeensis*	*A. americanum*
Heartwater	*Ehrlichia ruminantium*	*A. variegatum, A. hebraeum*
Bovine anaplasmosis	*Anaplasma marginale*	*Boophilus* spp.
Bovine babesiosis	*Babesia bigemina, B. bovis*	*Boophilus* spp.
Colorado tick fever	Reoviridae	*D. andersonii*
Rocky mountain spotted fever	*Rickettsia rickettsii*	*D. variabilis, D. andersonii*
Northern Asian tick typhus (Siberian tick typhus)	*Rickettsia sibirica*	*D. marginatus, D. silvarium, D. nuttalli*
Tick paralysis	Salivary toxin	*D. andersoni, D. variabilis, I. holocylus*
Crimean Congo hemorrhagic fever	Bunyaviridae	*Hyalomma marginatum, H. anatolicum, H. rufipes, H. marginatus*
Kyasanur forest disease	Flaviviridae	*H. spinigera*
Lyme disease	*Borrelia burgdorferi* sensu stricto	*I. ricinus* complex
American babesiosis	*Babesia microti*	*I. scapularis*
Human tick-borne ehrlichiosis	*Ehrlichia spp.*	*I. ricinus*
Tick-borne encephalitis	Flaviviridae	*I. ricinus, I. persulcatus, D. marginatus*
Epizootic bovine abortion	*B. coriaceae*	*Ornithodorus coriaceus*
Relapsing fever	*Borrelia recurrentis*	*Ornithodoros* spp.
Canine ehrlichiosis	*E. canis*	*Rhipicephalus sanguineus*
East Coast fever	*Theileria parva*	*R. appendiculatus*
Boutonneuse fever	*Rickettsia conorii*	*R. sanguineus, R. appendiculatus, Haemaphysalis leachi*

tum and the bont tick, *A. hebraeum* are also of medical and veterinary importance because they are the primary vectors of *Ehrlichia ruminantium* which causes 'Heartwater'. Heartwater is one of the more important cattle diseases in sub-Saharan Africa and Madagascar, and has recently appeared on a few islands in the Caribbean. The lone star tick, *Amblyomma americanum* is also of increasing importance due to changes in its geographical distribution, discovery of new pathogens for which it is a vector, and increased frequency of transmission of those zoonotic infectious agents to humans (Childs and Paddock 2003). *Amblyomma americanum* is the vector of *E. chaffeensis* which causes human ehrlichiosis. Multiple species of *Dermacentor* have also been implicated as major disease vectors in the USA and elsewhere. *Dermacentor andersonii* and *D. variabilis*, the Rocky mountain wood tick and the American brown dog tick, vector Rocky Mountain spotted fever, a disease caused by *Rickettsia rickettsii.*

8.1.3 Overview of Tick Biology

All ticks share the same basic developmental pattern; the egg hatches into a six-legged larva, which molts to an eight-legged nymph. Depending on the species, there may be one or multiple nymphal molts before the final molt to an eight-legged adult. Ticks, with rare exception, are obligate blood feeders at all life stages but are considered to be nonpermanent parasites in that they must find a new host each time they feed. Tick life cycles are defined by the number of hosts upon which a species will feed (Fig. 2). Argasid ticks feed on multiple hosts over a lifetime, even within a life stage and their most common hosts are generally small nesting vertebrates, such as birds and bats. In contrast, ixodid ticks will molt to the next life stage after each feeding on a host. In the Ixodidae, a mated female will deposit a single, large egg batch, and die shortly there-

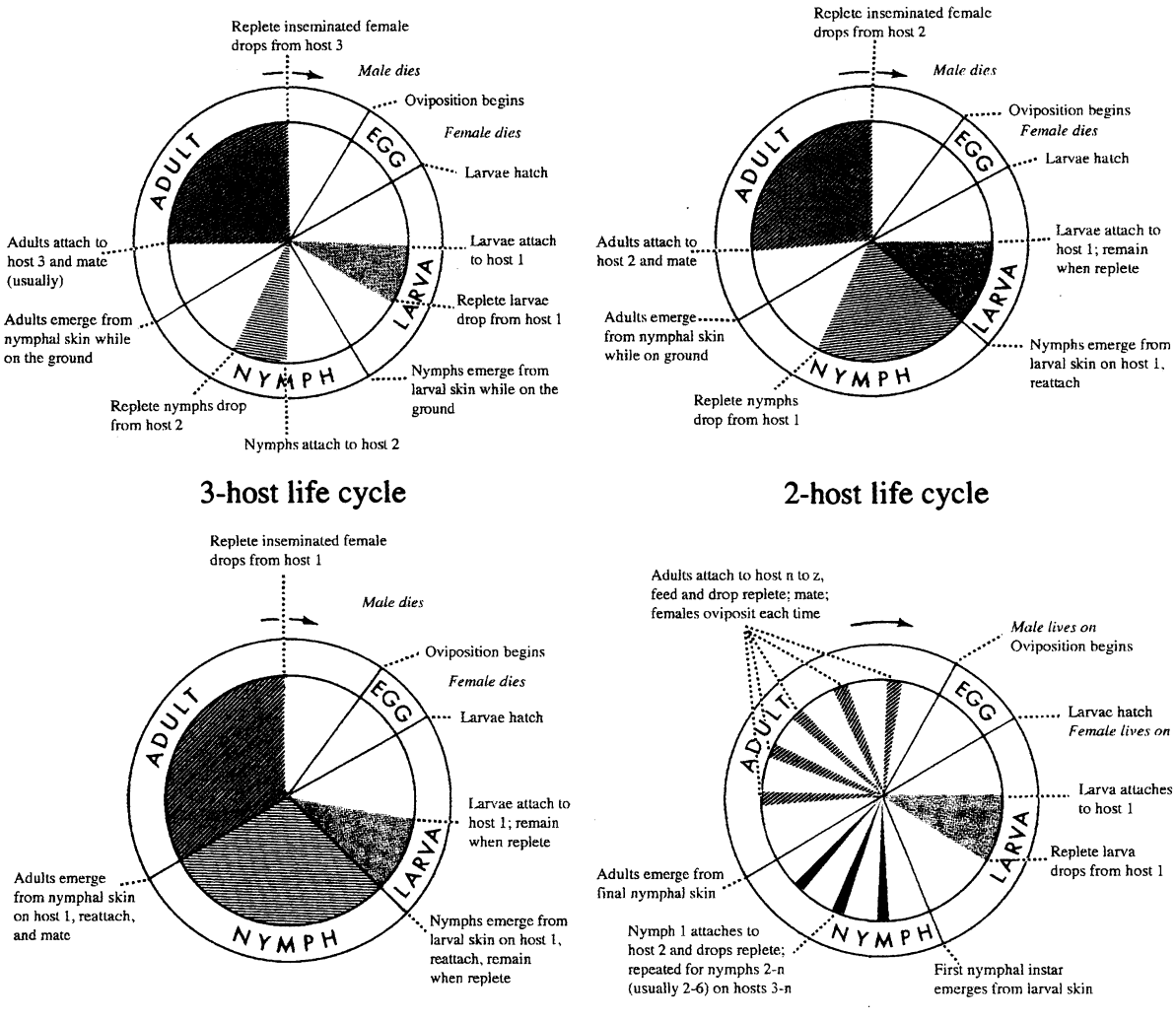

Fig. 2 Overview of life cycles observed in ticks. [Reprinted from Biology of Disease Vectors, 2nd edn, (eds) Marquardt WC, Black IV WC, Freier JE, Hemingway J, Higgs S, James AT, Kondratieff B, Moore C. Chap 4 Ticks, the Ixodida, p 50, Copyright (2005), with permission from Elsevier]

after. The eggs hatch into larvae, which begin active questing for a host. In "three-host" species such as *Ixodes, Amblyomma*, and some species of *Dermacentor*, larvae will attach and feed for 3–7 days. Once fully engorged, the larvae will drop off the host, molt to a nymph, and will then search for a new host. The nymph will feed for 3–8 days, drop off the host, molt to an adult, and seek a new host for a third time. The most common hosts of immature ixodid ticks are small mammals, ground dwelling birds, and lizards. Adult ixodid ticks tend to feed on larger mammals such as deer, livestock, dogs, and humans.

Depending on the species of tick, mating may occur on or off the host post-feeding. Some *Hyalomma* and *Rhipicephalus* species do not drop off after larval feeding, but instead molt on the host. This is considered a two-host life cycle. *Boophilus, Margaropus*, and some species of *Dermacentor* exhibit a one-host life cycle in which all stages of the tick remain on the

host from the first attachment until drop off as mated females.

Once a questing tick finds a host, and a suitable site to feed on the host, hard ticks penetrate the host skin with their chelicera and secrete a cement-like substance that helps to prevent detachment. Ticks imbibe the blood that pools in the wound site created by the mouthparts. Hemostasis is prevented by a mixture of several compounds present in the tick saliva (Ribeiro 1989, 1995), which are injected into the host by alternating cycles of feeding and salivating (Gregson 1967). Tick saliva also contains anti-inflammatory and immunomodulatory compounds that prevent immune reactions from disrupting the feeding process (Wikel 1999; Wikel and Alarcon-Chaidez 2001; Francischetti et al. 2005). While host immunity to salivary components may inhibit pathogen transmission, it has also been observed that the pharmacologic effects of tick saliva can enhance pathogen transmission (Gillespie et al. 2000).

8.1.4 Current Research Trends

Control of human tick transmitted diseases is difficult due to the lack of vaccines (Walker 1998; Dennis and Piesman 2005) and reliance on protective clothing, repellents, and tick checks (Ginsberg and Stafford 2005). Acaricides are the primary method for protecting livestock from tick infestation and tick-borne pathogens. However, the widespread development of acaricide resistance poses a serious challenge to effective control (Mitchell 1996; George et al. 2004). Development of novel control strategies depends on in-depth knowledge of tick biology and tick-host-pathogen interactions. While much progress has been made, significant gaps still exist in our understanding of many of these important and fundamental processes. An overview of the trends of current tick research is provided below, including specific areas that could likely benefit from advances in genetic and physical mapping.

We currently know very little of the mechanisms that allow one tick species to be a permissive vector and yet another refractory. Further elucidation of the molecular interactions that occur between the disease-causing agent and the tick during arthropod infection is needed. There has been considerable emphasis on the interaction between tick and vertebrate host, although surprisingly, not on the genetic basis for host preference and selection. Numerous tick salivary components have been characterized to identify pharmacologically active molecules (Ribeiro et al. 2006) as well as novel transmission blocking and anti-tick vaccine targets (Labuda et al. 2006). *Ixodes scapularis* saliva has been the most intensely studied producing a large annotated catalog of salivary transcripts (Ribeiro et al. 2006), and several specific proteins have been thoroughly described in function and structure. The proteins studied include enzymes, enzyme inhibitors, host protein homologs, immunoglobulin-binding proteins, amine-binding lipocalins, receptor agonist/antagonists, calcium-binding components, and cement cytokine components and an excellent review of 50 of these proteins is provided by Steen et al. (2006). An aspartic protease and a troponin-I-like molecule with anti-angiogenesis properties have been described in *Haemaphysalis longicornis*, a vector for a wide range of pathogens in East Asia and Australia (Boldbaatar et al. 2006; Fukumoto et al. 2006). Histamine release factors, which are critical to feeding, have been identified for *D. variabilis*, *D. andersoni*, *R. microplus*, and *A. americanum* (Mulenga and Azad 2005). Several tick proteins, including a histamine binding protein and a recombinant complement inhibitor are currently in clinical or preclinical human trials.

In recent years, the gene silencing technique of RNA interference (RNAi) has also been applied to understand the function of tick genes. Some of the first studies to silence expression of tick salivary gland transcripts using RNAi were demonstrated by Aljamali et al. (2002) and Narasimhan et al. (2004). More recently, RNAi has proved a rapid and cost-effective tool for screening large numbers of cDNAs to identify potential tick protective antigens (de la Fuente et al. 2005). Silencing of the anti-complement protein, *isac* in *I. scapularis* nymphs negatively affected tick feeding by reducing the fed tick weight by 40% (Soares et al. 2005). The causative agent of human granulocytic anaplasmosis, *Anaplasma phagocytophilum*, induces expression of the *salp16* gene in *I. scapularis* salivary glands during tick feeding. RNAi-induced silencing of *salp16* expression reduced the survival of *A. phagocytophilum* in infected mice (Sukumaran et al. 2006).

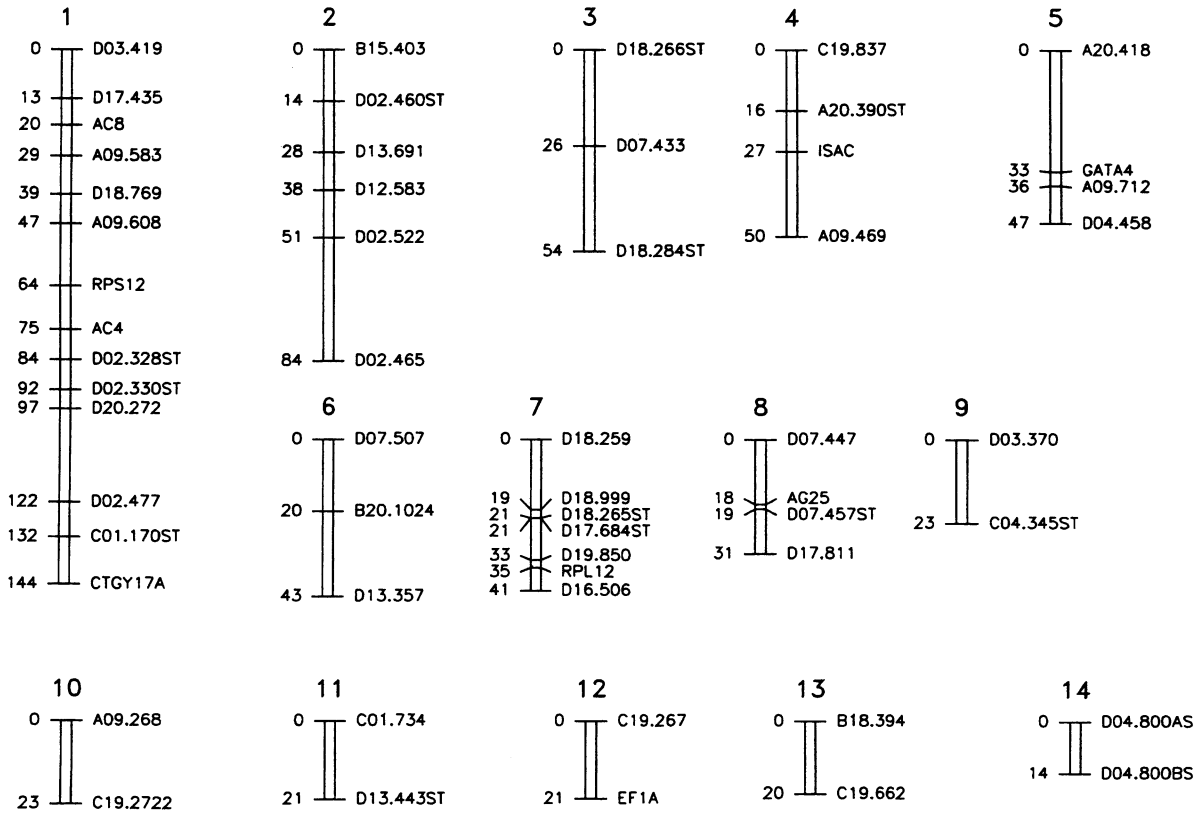

Fig. 3 Preliminary linkage map of the genome of *I. scapularis*. Corresponding mapped markers and accession numbers are shown in Table 2. [Reprinted from Insect Molecular Biology, vol 12, AJ Ullmann, J Piesman, MC Dolan, WC Black IV, A preliminary linkage map of the hard tick, *Ixodes scapularis*, pp 201–210, Copyright (2003), with permission from Blackwell]

Considerable research effort has also been focused on understanding the mechanisms of tick resistance to organophosphate (OP), synthetic pyrethroid, and amitraz acaricides. Both target site insensitivity and metabolic detoxification have been implicated as mechanisms of acaricide resistance in *R. microplus* (Jamroz et al. 2000). High frequencies of a phenylalanine to isoleucine amino acid substitution in domain III of a *para*-like sodium channel have been found in pyrethroid resistant strains of *R. microplus* (Guerrero et al. 2001). Several reports suggest the existence of multiple forms of acetylcholinesterase (AChE) with varying degrees of OP sensitivity (Reich et al. 1978; Wright and Ahrens 1988; Pruett 2002), but studies of putative AChE cDNAs cloned by a degenerate RT-PCR approach have failed to identify any resistance-associated sequence differences (Baxter and Barker 1998; Hernandez et al. 1999; Temeyer et al. 2004). Reciprocal cross experiments with resistant and susceptible *R. microplus* suggest that amitraz resistance in this species is inherited as an incomplete recessive trait (Li et al. 2005). An improved understanding of acaricide resistance in *R. microplus* and other tick pests is needed to delay the development of resistance to existing acaricides and to identify new acaricides with novel modes of action.

8.1.5 Classical Mapping Efforts

Many areas of tick research would benefit from classical mapping studies. However, one of the biggest impediments to the production of linkage maps for the Ixodida is the extremely long life cycle of some tick species, even when cultured under optimal laboratory conditions. As a consequence, the production of

Table 2 List of molecular markers and corresponding accession number (when available) mapped to the *Ixodes scapularis* genome

RAPDs	STARs	GenBank accession number	Microsatellites	GenBank accession number	cDNAs	GenBank accession number
A09.268	A20.390ST	BZ592382	ISAC4	AF331739-AF331739	EF1A	AF378368
A09.469	C01.170ST	BZ385517	ISAC8	AF331740	ISAC	AF270496
A09.583	C04.345ST	BZ385520	ISAG25	AF331742	RPL12	AAH8230
A09.608	D02.328ST	BZ385392	ISCTGY17A	AF331745-AF331747	RPS12	AF470687
A09.712	D02.330ST	BZ385393	ISGATA4	AF331753		
A20.418	D02.460ST	BZ385394				
B15.403	D04.800AST	BZ85433-BZ85434				
B18.394	D04.800BST	BZ85435-BZ85436				
B20.1024	D07.457ST	BZ385404				
C01.734	D13.443ST	BZ385415				
C19.267	D17.684ST	BZ385419				
C19.2722	D18.265ST	BZ385420				
C19.662	D18.266ST	BZ385421				
C19.837	D18.284ST	BZ385422				
D02.465						
D02.477						
D02.522						
D03.370						
D03.419						
D04.458						
D07.433						
D07.447						
D07.507						
D12.583						
D13.357						
D13.691						
D16.506						
D17.435						
D17.811						
D18.259						
D18.769						
D18.999						
D19.850						
D20.272						

a suitable F_1 backcross population is time consuming, laborious, and expensive. For example, *I. scapularis* has a two-year life cycle in the field although this can be shortened to approximately 9 months under optimal laboratory conditions. Another limiting factor is that few markers are available for map generation. To date, the only linkage map published for ticks is a preliminary map for *I. scapularis* developed by Ullmann et al. (2003) (Fig. 3).

8.2 Construction of Genetic Maps

The linkage map of Ullmann et al. (2003) was constructed based on segregation amongst 127 loci (Table 2). These included 84 random amplified polymorphic DNA (RAPD) markers, 32 sequence-tagged RAPD (STAR) markers, 5 cDNAs,

and 5 microsatellites in 232 F_1 intercross progeny from a single P_1 female collected from Bridgeport, Connecticut. Fourteen linkage groups were found, possibly reflecting the haploid number of chromosomes in *I. scapularis*. A preliminary map of 616 cM was generated with one marker every 10.8 cM. Given the genome size of *I. scapularis* (approximately 2.1–2.3 Gbp), the relationship of physical to genetic distance is estimated to be approximately 663 kb/cM (2.1×10^3 Mbp/3,166 cM).

Sequence-tagged RAPD markers were the most useful of all the markers analyzed in the Ullmann et al. (2003) study. Of the 65 primer sets designed for STARs, 52 were polymorphic with 37 conforming to mendelian ratios. Markers derived from simple sequence repeats (SSR) or microsatellites are highly desirable because they are based upon DNA polymorphisms and are co-dominant. Unfortunately, microsatellites were not as productive for generating markers in *I. scapularis*. The reasons for this are still unclear but may be associated with the organization of short sequence repeats in the genome (see discussion below). Characterization of microsatellites, or the lack thereof, in *I. scapularis* has been described in earlier work (Fagerberg et al. 2001).

From the 20 RAPD primers identified by Ullmann et al. (2003), a total of 63 markers were mapped to the *I. scapularis* genome (Table 2). This is in stark contrast to 94 markers generated with 10 RAPD primers in *Aedes aegypti* (Antolin et al. 1996) and once again, may reflect the organization of repetitive DNA in the genome. The advantage of RAPD-PCR is that it requires no sequence information and is thus especially useful in the absence of genomic sequence. In addition, each RAPD-PCR primer can potentially reveal several usable loci. A drawback is that RAPD-PCR generates dominant markers which hinder phase determination, or identification of the origins of a genotype in the F_2 and some backcross genotypes. Since phase determination is essential to estimate recombination frequencies, dominant markers are of limited utility in linkage mapping. STARs were used for *I. scapularis* to address the phase determination problem because this method converts dominant into co-dominant bands (Bosio et al. 2000).

Tick research would greatly benefit from linkage maps for a range of tick species. Additional markers are also needed to develop a second generation, high-resolution map for *Ixodes*. Such maps would have enormous utility for map-based (positional) cloning of genes of interest, particularly as so few tick genes have been characterized to date. The drawback is the large number of markers that will be required to adequately map these organisms, especially given the genome size of many ixodid ticks (see below). One benefit of tick genomic sequence will be the opportunity to identify additional, potentially polymorphic markers such as microsatellites and single nucleotide polymorphisms (SNPs) in *Ixodes* and other ticks. cDNA sequence can also be used to generate polymorphic markers (Fulton et al. 2001) and could potentially provide a solution to this problem; however, large introns in *I. scapularis* and possibly other tick species may make this methodology horribly expensive and restrictive.

8.3 Efforts in Forward Genetics

The fact that ticks are not genetically tractable has undoubtedly proven a massive impediment to forward genetics studies of the Ixodida. Methods for tick mutagenesis and genetic screening have not been developed and there are currently no phenotypic and few molecular markers available for ticks. The majority of these have been developed in *Ixodes* and *Rhipicephalus* species (reviewed by Navajas and Fenton 2000). To date, the only approach to investigate gene function in ticks is the reverse genetics method employing RNAi (reviewed above).

8.4 Mapping of Quantitative Trait Loci

High-density linkage mapping of a genome provides an opportunity to identify the genes which affect quantitative traits. There is a real need to identify quantitative trait loci (QTL) associated with phenotypes such as tick vector competence, host seeking and specificity, and acaricide resistance. Currently, QTL mapping is not possible for any tick species, due to the lack of high-density linkage maps. Unfortunately, tick QTL mapping is also limited by the lack of tick populations that are either permissive or refractory for a specific phenotype of interest. However, with

a tick genome sequence in hand for *Ixodes*, it is now incumbent on the tick research community to develop the necessary markers and strains for QTL mapping in this and other tick species of medical and veterinary importance. The development of *Ixodes* strains that are refractory and susceptible to key pathogens, or that differ in their preference for a vertebrate host as a blood meal source would be an important investment for scientists. Strains of *R. microplus* with resistance to one or several classes of acaricides are available and a number of these are maintained by the United States Department of Agriculture (USDA); such populations may find application in the mapping of resistance traits.

8.5 Advanced Work

8.5.1 Physical Mapping Efforts

The ultimate goal of any genome sequencing project is the development of a physical map of the genome in which the order of every base within the genome and its location on chromosomes is known. Physical maps provide the framework needed to identify expressed, non-expressed, repetitive, unique non-coding, structural, and regulatory sequences from raw sequence data. Unfortunately, with the exception of rudimentary karyotyping by Oliver (1977) and Hilburn et al. (1989), nothing regarding tick chromosome structure and genome organization is known. Until recently, physical mapping techniques such as fluorescence in situ hybridization (FISH), widely used in many genome efforts (Adams et al. 2000; Holt et al. 2002; Hong et al. 2003) to assign and orient scaffolds on individual chromosomes, have not been developed for the Ixodidae.

The chromosomal organization of the *R. microplus* genome is currently being resolved by applying FISH to testicular chromosome preparations (C. Hill personal communication). Unfortunately, despite analysis of numerous tissues, researchers have failed to identify polytene chromosomes in the ticks. However, *R. microplus* cells in meiosis I provide a resolution that is sufficient for detailed observations. A combination of FISH, chromosome morphology, and relative chromosome length has made it possible to identify the X chromosome and specific autosomes (2–10) in

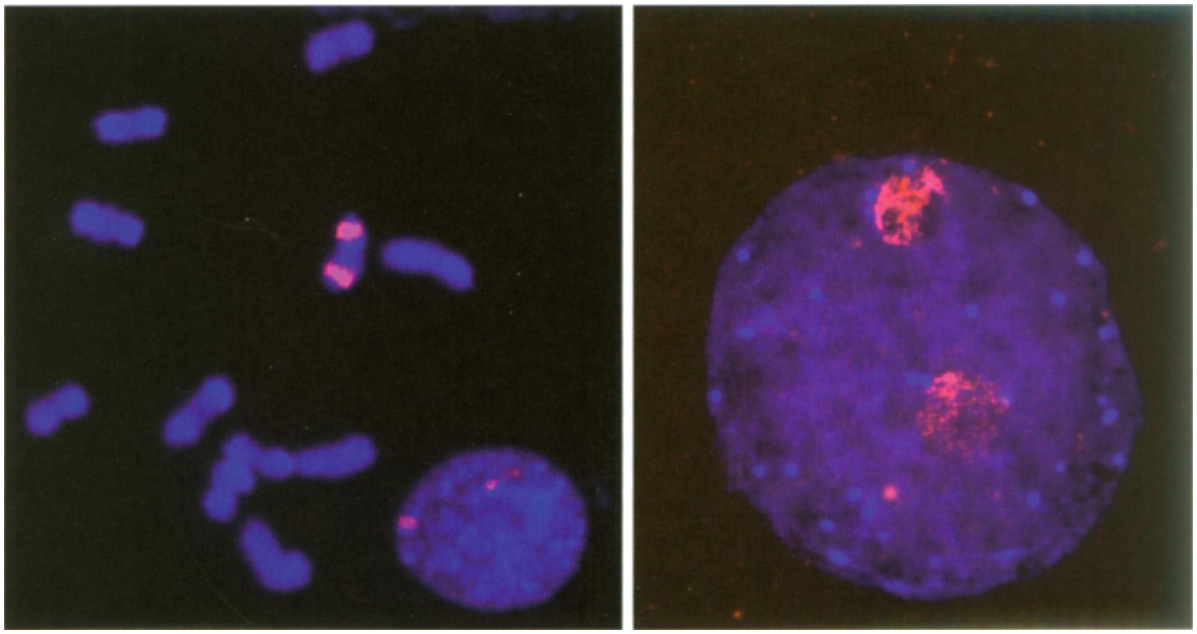

Fig. 4 Fluorescence in situ hybridization (FISH) mapping of an rDNA probe (red label) to *R. microplus* meiotic chromosomes (*left*) and diploid interphase nucleus (*right*)

metaphase I preparations from *R. microplus* (Fig. 4, left panel). The chiasmata associated with each autosome pair are visible in preparations as the chromosomes take the end-to-end formation typical of holocentric chromosomes. Hybridization of the ribosomal DNA (rDNA) repeat on chromosome 6 confirms this orientation and holocentric nature. Heterochromatic termini have also been discovered containing highly repetitive DNA sequence. These knobs are visible in interphase cells as condensed spots (Fig. 4, right panel) that are likely chromomeres. Tandem repeats of approximately 150 bp have been localized to these possibly telomeric regions (N. Geraci unpublished). The quantity of hybridization of these sequences on each chromosome is used as an aid in chromosome (bivalent) identification.

This work represents an important advance for tick genomics and genetics. Such techniques must now be established for *I. scapularis* in order to assemble and mine the genome for sequences of interest. Physical mapping will also permit invaluable studies of tick chromosome biology and population genetics. Physical mapping techniques and resources such as bacterial artificial chromosome (BAC) libraries must now be developed for other tick species of economic importance.

8.5.2 Sequencing Projects: ESTs and Whole-genome Shotgun Sequencing

Historically, expressed sequence tag (EST) sequences have proven invaluable for tick gene discovery, especially in the absence of genomic sequence. A review of the NCBI dbEST database reveals approximately 64,000 EST sequences for both pro- and metastriate species including *Amblyomma, Boophilus, Rhipicephalus, Dermacentor,* and *Ixodes* species. Libraries produced from tick salivary gland, midgut and ovary tissues have proved popular (Santos et al. 2004; Ribeiro et al. 2006) as have studies of tick expression profiles pre-, during, and post-blood meal and pre- and post-infection with various pathogens. The most comprehensive *R. microplus* EST study to date was undertaken by Guerrero et al. (2005) who identified 8,270 unique tentative consensus (TC) and singleton sequences from 20,417 EST sequencing reads of an *R. microplus* pooled tissue library. The Institute for Genomic Research (TIGR) auto-annotation pipeline was employed to assign putative function to these TCs and to create a gene index for several tick species (http://www.compbio.dfci.harvard.edu/tgi).

Expressed sequence tags will continue to be an invaluable tool for tick research and genome annotation especially. Firstly, the evolutionary distance between the subphylum Mandibulata and Chelicerata (Klompen et al. 1996) will likely restrict the identification of tick genes based on homology to insect sequences. The fact that approximately 45% of tick ESTs have no matches in sequence databases (Hill and Gutierrez 2000; Nene et al. 2002; Valenzuela et al. 2002; Guerrero et al. 2005) illustrates this point. Secondly, tick genome annotation will likely be complicated by large introns and significant amounts of repetitive DNA (discussed below).

Several studies have provided an insight into the size and organization of tick genomes and are an important precursor to large-scale genome sequencing. Genome size is an important consideration for sequencing projects because it determines the amount of sequencing that must be undertaken and thus ultimately the cost of a genome project. Reassociation kinetics has been used to determine the genome size of three species of hard ticks. Ullmann et al. (2005) estimated the genomes of *I. scapularis* and *R. microplus* to be approximately 2.15 pg (2.1 Gbp) and 7.5 pg (7.1 Gbp), respectively. The genome of *A. americanum* was reported as 1.08 pg (1.04 Gbp) (Palmer et al. 1994). In other work, an extensive analysis of genome size in multiple species of Ixodida was conducted using the technique of flow cytometry (N. Geraci personal communication). Results suggest a haploid genome size of >1,000 Mbp for all Ixodida examined, with a mean of 1,252 Mbp (1.28 pg) for the Argasidae and 2,610 Mbp (2.67 pg) for the Ixodidae. Estimates for *I. scapularis* compared favorably with that of Ullmann et al. (2005). It would appear that the hard and soft tick species examined to date have genomes that are significantly larger than any sequenced invertebrate. Large genome size in the Ixodida is likely due to the accumulation of non-coding, repetitive DNA. The reasons for this are unclear but could involve the accumulation of transposable elements, segmental duplications and simple sequence repeats, an increase in intron size, and possibly one or multiple polyploidy events.

The above-mentioned studies suggest that moderately repetitive sequences constitute a greater percentage of the *R. microplus*, *I. scapularis*, and *A. americanum* genomes than either highly repetitive or unique sequences (Palmer et al. 1994; Ullmann et al. 2005). Furthermore, *R. microplus* and *I. scapularis* both exhibit a mix of short and long period interspersion (Ullmann et al. 2005), a characteristic of large vertebrate genomes. Additionally, highly repetitive sequences in the *R. microplus* and *I. scapularis* genomes were reported to be of relatively low complexity (Ullmann et al. 2005), suggesting the presence of many simple sequence repeats. These findings have important implications for tick genomics research; extensive sequencing of these and possibly other ixodid ticks will likely be necessary in order to identify unique, presumably coding sequence. Furthermore, significant amounts of repetitive DNA may present a challenge for genome assembly and annotation.

Several tick species have been considered for a genome sequencing effort. Large-scale sequencing projects are underway for both a pro- and a metastriate species. Foremost amongst these is the *Ixodes scapularis* Genome Project (IGP), the goal of which is to sequence the genome of a medically significant tick. The IGP was approved by the National Institutes of Health (NIH) in 2004 (Hill and Wikel 2005). This project will sequence the *I. scapularis* genome to a 6× level of coverage; it is the first to sequence a tick and a member of the subphylum Chelicerata. The project is a partnership between the NIH, the tick research community, the J. Craig Venter Institute (JCVI) and the Broad Institute. As of September 2007, over 19 million trace reads representing approximately 10.4×10^{10} bp of *I. scapularis* DNA have been generated, the equivalent of more than 6× coverage of the genome. A 10× *I. scapularis* BAC library has been generated to facilitate genome assembly and BAC-end sequencing of these clones is ongoing. Over 200,000 *I. scapularis* ESTs have also been sequenced for gene discovery and annotation. Genome assemblies and automated annotations will be made available through public databases maintained by the National Center for Biotechnology Information (NCBI), the JCVI, and Broad sequencing centers as well as VectorBase (http://www.vectorbase.org/index.php; Lawson et al. 2007), the NIH-supported bioinformatics database for invertebrate vectors of human disease.

There is also considerable interest in an *Rhipicephalus microplus* genome sequencing project and USDA scientists have developed a number of resources toward such an effort. Over 40,000 ESTs have been generated and a 1× BAC library is also available. In preliminary work, investigators have been able to assemble a 120-kbp insert BAC from BAC shotgun reads (F. Guerrero personal communication) suggesting that assembly of regions of the *R. microplus* genome will likely be feasible. More recently, a whole-genome shotgun sequencing project was approved for another member of the subclass Acari, namely the two-spotted spider mite *Tetranychus urticae* (M. Navajas personal communication). This project may be of particular advantage to the *I. scapularis* genome project. At 79 Mbp, the *T. urticae* genome is estimated to be one of the smallest invertebrate genomes and, as such, may be an extremely useful model for assembly and annotation of larger tick genomes. Furthermore, comparative analyses between haematophagous tick vectors and the plant phytophagus *T. urticae* may aid the identification of genes associated with parasitism and pathogen transmission.

The *I. scapularis* and *R. microplus* genomes represent a tremendous resource for tick and tick-borne disease research. In addition to informing on many aspects of tick biology, sequence data may permit the identification of new targets for vaccine and acaricide development. These genomes will also facilitate comparative genomic analyses with a plethora of sequenced organisms, revealing fundamental differences in the genomes of tick and insect disease vectors as well as between the pro- and metastriate ticks. As such, they offer the opportunity to understand the genetic level differences associated with aspects of tick biology such as host preference, reduction in number of hosts, and vector competence between three- and one-host ticks. They will also offer insights into the architecture, organization, and regulation of large, repetitive tick genomes and may help to resolve questions of Ixodida phylogeny and evolution.

8.6 Integration of Genome Information and Future Work

Tick genomics will present scientists with unprecedented opportunities and unique challenges. Some

of these opportunities are outlined above and many more will likely become apparent as scientists begin to interrogate genomic sequence. Unfortunately, the size of tick genomes and their repetitive DNA content may be problematic, particularly for genome assembly and annotation. Given the imminent release of the assembled *Ixodes* genome, scientists must be prepared to address such challenges in order to fully exploit the *Ixodes* sequence and ultimately advance tick genomics research.

Physical mapping will be essential for assembly of the *Ixodes* genome as well as for population genetics studies. FISH mapping techniques developed for *R. microplus* must now be expanded to *Ixodes* and a range of other ticks that impact human and animal health. The IGP will generate the first physical map of a tick and possibly a chelicerate genome. As such it will provide an insight into the nature and organization of coding and non-coding DNA in the genome. The automated and manual annotation of genes based largely on sequence homology will not be sufficient to catalog the entire repertoire of *Ixodes* genes and transcripts or to identify the genes associated with specific phenotypes of interest. Additional markers and a high-resolution genetic map of *I. scapularis* will be essential for integration of the *Ixodes* genetic and physical maps as well as to facilitate QTL mapping in this vector.

Repetitive DNA and divergence between ticks and insects may necessitate the development of specific assembly and gene prediction software for tick genomics. Scientists will also need to acquire expertise in the manual annotation of tick sequence. Additional EST sequences from tissue-specific libraries and from a range of tick and chelicerate species will be invaluable for the identification of low abundance transcripts, genome annotation, and comparative analyses. Microarrays, widely used for transcription profiling in a number of invertebrates, are an essential tool that must be developed by the tick research community. RNAi of several tick genes has proved successful and this technique must now be expanded for functional analysis of predicted genes.

Genome size and sequencing costs may prohibit the sequencing of additional tick species of medical and veterinary importance in the immediate future. Consequently, comparative analyses between tick species will likely prove invaluable until such time as genome sequencing becomes more cost-effective. In the interim, the development of genetic and physical maps and associated resources is essential in order to realize the full potential of available and anticipated genomic data.

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