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Variation in genome size of argasid and ixodid ticks

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Abstract

The suborder Ixodida includes many tick species of medical and veterinary importance, but little is known about the genomic characteristics of these ticks. We report the first study to determine genome size in two species of Argasidae (soft ticks) and seven species of Ixodidae (hard ticks) using flow cytometry analysis of fluorescent stained nuclei. Our results indicate a large haploid genome size ($1C > 1000$ Mbp) for all Ixodida with a mean of 1281 Mbp (1.31 ± 0.07 pg) for the Argasidae and 2671 Mbp (2.73 ± 0.04 pg) for the Ixodidae. The haploid genome size of *Ixodes scapularis* was determined to be 2262 Mbp. We observed inter- and intra-familial variation in genome size as well as variation between strains of the same species. We explore the implications of these results for tick genome evolution and tick genomics research.

Keywords: Ixodidae; Argasidae; Flow cytometry; Genome size; *C* value

1. Introduction

Ticks (subphylum Chelicerata, class Arachnida, subclass Acari, superorder Parasitiformes; suborder Ixodida) are of global medical and veterinary importance due to their ability to transmit a greater variety of arboviruses, bacteria, and protozoa than any other blood feeding arthropod (Balashov, 1972; Jongejan and Uilenberg, 2004; Dennis and Piesman, 2005). Other forms of injury attributed to ticks include anemia, dermatosis, toxemia and paralysis (Sonenshine, 1991; Roberts and Janovy, 1996). Worldwide, tick-borne infectious diseases are emerging and resurging (Walker, 1998; Telford and Goethert, 2004; Walker, 2005). In the USA, the resurgence of Lyme disease and the emergence of other tick-borne diseases such as human granulocytic anaplasmosis (HGA) (Gratz, 1999; Childs and Paddock, 2003) vectored by species of *Ixodes*, pose increasing public health concerns. 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; Mans et al., 2004). 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, repellants and tick checks (Ginsberg and Stafford, 2005). Acaricides are the primary method for protecting livestock from tick infestation and tick-borne pathogens, but the widespread development of resistance to these control chemicals 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 host–tick–pathogen interactions, but significant gaps exist in our understanding of many of these important and fundamental processes.

The tools of genomics and proteomics provide powerful approaches for understanding the biology of disease vectors and the relationships of those arthropods with the infectious agents they transmit. Vector genome sequencing provides insights into the full spectrum of

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genes associated with important aspects of vector biology (Hill et al., 2005; Hill and Wikel, 2005). The first vector genome to be sequenced was that of the African malaria mosquito, *Anopheles gambiae* (Holt et al., 2002). This project has provided novel insights into the mechanisms of mosquito host location, insecticide resistance and vector–pathogen interactions (Hill et al., 2005). More recently, the genome of the yellow fever mosquito, *Aedes aegypti*, has been sequenced and assembled and other vector genome projects are planned or in progress. The *Ixodes scapularis* Genome Project (IGP) was initiated in 2004 to sequence the genome of *I. scapularis* (Lyme disease tick) to a draft level of coverage (Hill and Wikel, 2005). The IGP is a collaboration between the National Institutes of Health and the international tick research community. It is the first to investigate a tick of medical significance and whole genome shotgun sequencing is currently ongoing. During recent years, little research was devoted to determine the genetics and genomics of the two major families of ticks, namely the Ixodidae (hard ticks) and Argasidae (soft ticks) and further research is required to broaden our understanding of tick biology, vector competence, and disease–host relationships. Assessment of tick genome size is a useful starting point to spur further genomics research of the Ixodida.

The haploid genome size of an organism, or "*C*-value", is the total amount of DNA contained in the haploid set of chromosomes (Swift, 1950). Genome size varies greatly among taxa and even within genera (Mirsky and Ris, 1951; Gregory, 2005a). An estimate of genome size can provide insight into important characteristics of the genome, such as the amount of repetitive and unique, presumably coding sequence. Genome size is also an important consideration for large scale genomic sequencing projects because it determines the amount of sequencing that must be undertaken, and ultimately the cost of a genome project.

Historically, methods for determining genome size have included Feulgen densitometry and reassociation kinetics (Leroy et al., 2003). Densitometry relies on measurements of light absorption by stained nuclei to estimate genome size. Many hundreds of nuclei must be assessed per sample and differences in slide preparation can lead to significant variation between measurements. Reassociation kinetics is widely used and entails shearing, denaturing, and re-annealing of genomic DNA (Ullmann et al., 2005). The rate of re-annealing is used to estimate genome size by comparison to a known standard. Reassociation kinetics has been used to determine the genome size of three ixodid tick species to date. The genomes of *I. scapularis* and *Rhipicephalus* (*Boophilus*) *microplus* (hereafter *B. microplus*) are reported to be approximately 2.15 pg (2.1 Gbp) and 7.5 pg (7.1 Gbp), respectively (Ullmann et al., 2005). Palmer et al., (1994) estimated the genome of *A. americanum* to be 1.08 pg (1.04 Gbp). One advantage of reassociation kinetics is that it can contribute information on genome composition such as the percentage of fold back (tandem inverted repeats), highly repetitive, moderately repetitive and unique sequence in a genome. Unfortunately, reassociation kinetics tends to be both laborious and time consuming and the number of samples that can be analyzed at any one time is limited (Leroy et al., 2003). Moreover, studies suggest that reassociation kinetics may underestimate genome size (Bennett et al., 2003; Schmuths et al., 2003; Dolezel and Bartos, 2005).

Flow cytometry is a method to quantify the number and relative size of cells or cellular components based on the fluorescence intensity of fluorochrome-labeled material (Cram, 2002). Flow cytometry of fluorescently stained nuclei has been used to determine the genome size of a number of organisms, including bacteria (Button and Robertson, 2001), mammals (Ibrahaim and van den Engh, 2004), plants (Bennett et al., 2000; Bennett et al., 2003), and nematodes (Leroy et al., 2003) and more recently, several arthropod species (Marescalchi et al., 1990; Schlipalius et al., 2002; Bennett et al., 2003; Johnston et al., 2004). Fluorescence emission intensity of a stained DNA sample, relative to that of an internal standard of known genome size, can be used to estimate the total amount of DNA per cell (Price and Johnston, 1996; Bennett et al., 2003; DeSalle et al., 2005). The advantage of flow cytometry over other methods for estimating genome size is that it is rapid, more precise and requires less cellular material (Galbraith et al., 1983; Hardie et al., 2002; Bennett et al., 2003; Leroy et al., 2003). In addition, studies have shown that *C*-value estimates obtained by flow cytometry are in close agreement with values obtained from complete genome sequencing (Bennett et al., 2003).

This study is the first to estimate genome size across the suborder Ixodida; our analysis includes subfamilies within the Argasidae and Ixodidae and representatives of the pro- and metastriate lineages. We also analyzed inter- and intra-familial variation in genome size as well as variation between strains and sexes of the same species. These results expand our knowledge of tick genome size, confirm some previous estimates and differ from earlier estimates of *A. americanum* genome size.

2. Materials and methods

2.1. Tick colonies

Adult male and female *Amblyomma cajennense* (Cayenne tick), *Amblyomma maculatum* (Gulf Coast tick), *Argas brevipes*, and *Ornithodoros turicata* (relapsing fever tick) maintained at the Department of Entomology, Texas A&M University as described by Strey et al. (2001) were obtained from P. D. Teel. Adult male and female *A. americanum* (lone star tick), *Dermacentor variabilis* (American dog tick), *Dermacentor andersoni* (Rocky Mountain wood tick), *Ixodes pacificus* (western black-legged tick) and *I. scapularis* (black-legged tick) were acquired from colonies maintained at the University of Connecticut Health Center (UCHC) as described by Bouchard and Wikel (2005). Immature tick stages (larvae

Table 1
Genome size of ticks in the families *Ixodidae* and *Argasidae*

Species, sex	2C GS±SE (pg)	1C GS±SE (Mbp)	*N*	Sex system	2n
Family ixodidae (hard ticks)					
Amblyomma americanum, ♀	6.36±0.06	3108±27	10	XX	22
Amblyomma americanum, ♂	5.31±0.06		11	X0	21
Amblyomma cajennense, ♀	5.68±0.03	2779±18	11	XX	22
Amblyomma cajennense, ♂	4.86±0.14		10	X0	21
Amblyomma maculatum, ♀	6.03±0.05	2949±24	12	XX	22
Amblyomma maculatum, ♂	5.20±0.08		12	X0	21
Dermacentor andersoni, ♀	5.61±0.12	2744±59	11	XX	22
Dermacentor andersoni, ♂	5.05±0.08		11	X0	21
Dermacentor variabilis, ♀	5.85±0.16	2862±79	11	XX	22
Dermacentor variabilis, ♂	5.51±0.14		11	X0	21
Ixodes pacificus, ♀	4.08±0.03	1993±13	4	XX	28
Ixodes pacificus, ♂	3.95±0.20		4	XY	28
Ixodes scapularis, ♀	4.63±0.05	2262±27	16	XX	28
Ixodes scapularis, ♂	4.85±0.05		16	XY	28
Family argasidae (soft ticks)					
Argas brevipes[a]	3.01±0.03	1472±13	9	NA	NA
Ornithodoros turicata[a]	2.23±0.25	1090±120	4	NA	NA

The average diploid (2C) and haploid (1C) genome size (GS) and the species, sex, number of individuals sampled (*N*), sex chromosome system, and 2n number of chromosomes are shown. The sex determination system and 2n chromosome number are as reported by Gunn and Hilburn (1995) for *A. maculatum* females and Oliver et al. (1982, 1993) for all other species.

[a]These data likely represent combined estimates from males and females.Mbp, mega basepairs; NA, not available; pg, picograms; SE, standard error.

and nymphs) were fed on mice and adult ticks were fed on rabbits. The *I. scapularis* (Wikel strain) colony is the reference strain for the IGP. The *D. andersoni* Montana, ID, and LG6OSU strains, originally established from a founder population collected in the Lake Como/Ravalli County area of western Montana (Montana strain), a colony maintained originally by the United States Department of Agriculture (USDA) at the University of Idaho (ID strain), and a colony maintained by Oklahoma State University (LG6OSU strain) and maintained as separate lineages, were obtained from UCHC. *D. andersoni* genome size estimates reported in Table 1 were obtained using the Montana strain.

2.2. Sample preparation

Tick nuclei were prepared from whole tick specimens using a modified version of DeSalle et al. (2005). Briefly, male and female ticks were separately dissected under modified Galbraith buffer (30 mM $Na_3C_6H_5O_7.2H_2O$; 18 mM MOPS with sodium salt; 21 mM $MgCl_2.6H_2O$; 0.1% Triton X-100; 1 μg/ml RNase A) (Galbraith et al., 1983). Synganglia (brains) were dissected using a No. 11 scalpel and separately transferred to a 2 mL Kontes Dounce homogenizer vial containing 1 mL of Galbraith buffer. Tissue was ground 10 times with steady pressure using an "A" pestle to separate nuclei from cells. The suspension was then filtered through 20 μm nylon mesh (Small Part, Inc. Miami Lakes, FL) into a 1.5 mL microcentrifuge tube. A suspension of *Gallus domesticus* (white leghorn chicken) red blood cells (CRBCs) was added to each sample as an internal standard to achieve a final concentration of 1.5×10^6 CRBCs/mL and the suspension was mixed by gentle inversion. Propidium iodide (PI) was added to each sample to a final concentration of 75 μM and allowed to bind to DNA for at least 20 min in the dark at 4 °C. This process was replicated for each sample (see Table 1 for the total number, *N*, of each sex and species analyzed).

2.3. Flow cytometry

Samples of stained tick nuclei suspensions were analyzed on a Cytomics FC 500 flow cytometer (Beckman-Coulter, Fullerton, CA), using an air-cooled argon-ion laser tuned to 488 nm with 20 mW output. Via the use of core flow stream of 1X PBS, nuclei were analyzed as they passed through the laser in single file. Propidium fluorescent emissions of laser-excited nucleic material were recorded by a photomultiplier tuned to collect a spectral band of 20 nm with a full-width half-maximum at 675 nm. To avoid bias in the data, large outliers of forward and side scatter signals were excluded by gating (Fig. 1(a)). The events at particular levels of propidium fluorescence were used to generate a histogram (Fig. 1(b)). The mean fluorescence of CRBC and tick 2*C* nuclei was calculated by separately gating the respective sample signals on the histogram (Fig. 1(a)). On average, approximately 2000 nuclei were counted per sample.

2.4. Calculation of genome size

Estimations of average genome size were made among nine species of Ixodida, with seven members of the Ixodidae (two prostriates and five metastriates) and two members of the Argasidae (Table 1). The genome size of three strains of *D. andersoni* was also determined (Table 2). The mean 2*C* value of multiple individuals per sex of each species was determined, with the exception of the Argasid ticks. Males and females of these species could not be separated because they do not exhibit sexual dimorphism. Thus our genome size estimations for *A. brevipes* and *O. turicata* are likely an average of measurements from both males and females. The amount of nucleic material in each tick sample was estimated by comparison to the CRBC internal standard. For the purposes of this study, we used a CRBC 2*C* value of 2.33 pg of DNA, as described by Bennett et al. (2003) and Galbraith et al. (1983). The diploid genome size of each sample was calculated in pg of DNA using the conversion of Dolezel et al. (2003), where 1 pg DNA = 0.978×10^9 bp. Average haploid 1*C* genome size was calculated from diploid 2*C* female values only. 1*C* values were not calculated for males because males are either XY or X0. We used a standard Student's *t*-test, with a *p*-value of $\leqslant 0.01$ to determine significant differences in

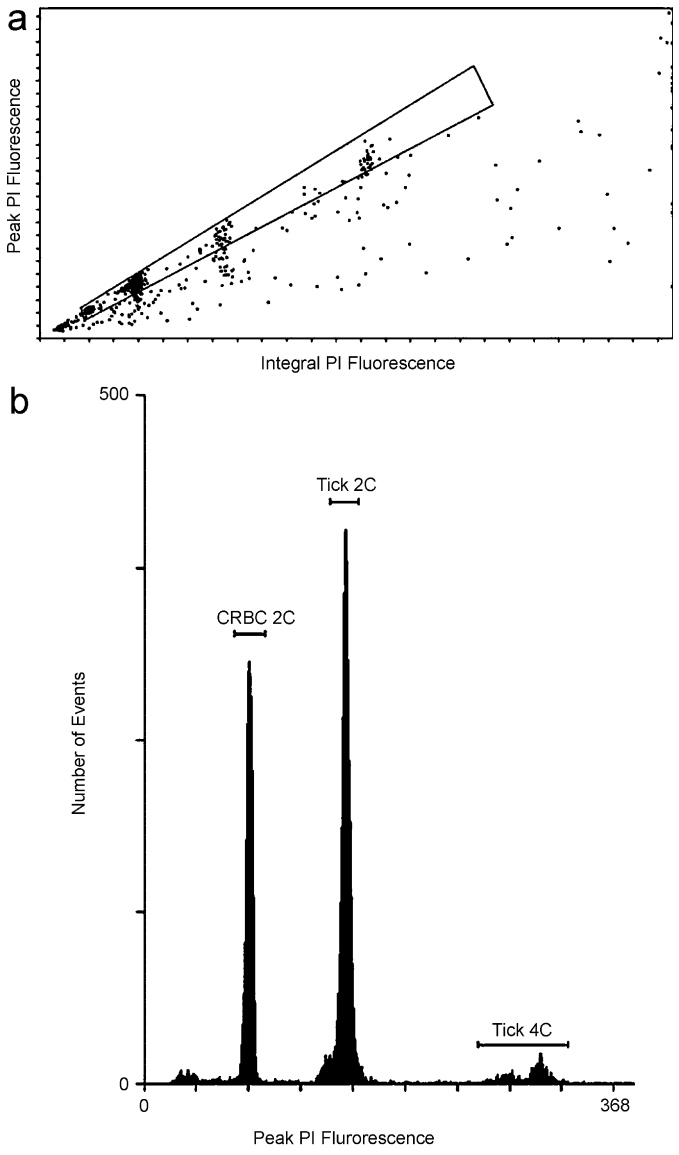

Fig. 1. Flow cytometry analysis of propidium iodide stained nuclei prepared from a single female *Ixodes scapularis* synganglion and chicken red blood cells (CRBCs; internal standard). (a) Dotplot of peak PI fluorescent signals versus integral or total PI fluorescent signals for each event with gate lines indicating events included in analysis and (b) histogram of propidium iodide fluorescent signals for the same sample showing *I. scapularis* 2C and 4C peaks and CRBC 2C peak. PI, propidium iodide.

genome size between species within a genus, strains of the same species, and sexes of the same strain (Table 3).

2.5. Genome size comparisons and phylogenetic analysis

The haploid genome size of tick species investigated in this study was compared to 1*C* values reported for species of Arthropoda (Fig. 2). Tick genome size was mapped onto a proposed phylogeny of the suborder Ixodida (Fig. 3) developed based on Balashov (1972), Klompen et al. (2000) and Barker and Murrell (2004).

Table 2
Genome size of male and female *Dermacentor andersoni* strains (Montana, ID and LG6OSU)

Strain, sex	2C GS±SE (pg)	1C GS±SE (Mbp)	*N*
Montana, ♀	5.61±0.12	2744±59	11
Montana, ♂	5.05±0.08		11
ID, ♀	5.84±0.07	2855±36	5
ID, ♂	5.46±0.04		5
LG6OSU, ♀	5.36±0.04	2623±20	3
LG6OSU, ♂	5.15±0.04		7

The strain, sex, average diploid (2C) and haploid (1C) genome size (GS), and the number of individuals sampled (*N*) are shown. Mbp, megabasepairs; pg, picograms; SE, standard error.

3. Results

Fig. 1(a) is an example dot plot showing the peak propidium fluorescence emission (the strength of each signal) vs. the integrated propidium iodide fluorescence (the total amount of signal per event) for each event recorded for a suspension of PI stained nuclei prepared from the synganglion of a single female *I. scapularis* and CRBCs. The histogram corresponding to this sample is provided in Fig. 1(b), showing the observed numbers of events (*y*-axis) versus PI fluorescence emission (*x*-axis) recorded for each event.

Table 1 shows the average diploid (2*C*) and haploid (1*C*) genome size for females and the diploid genome size of males of each species of tick sampled in this study. CRBCs are an appropriate standard firstly because they are non-dividing (and hence mostly 2*C*) and secondly because the *G. domesticus* genome is similar in size to published tick *C* values (Palmer et al., 1994; Ullmann et al., 2005). Diploid genome size estimates for the argasid species, *A. brevipes* and *O. turicata,* likely represent an average of values for both males and females because adults of these species cannot be sexed based on external morphology. We calculated haploid (1*C*) genome size using female 2*C* estimates only because (i) the diploid number of chromosomes is odd in the males of species with X0 sex systems (*A. americanum*, *A. maculatum*, *A. cajennense*, *D. andersoni*, and *D. variabilis*), and (ii) the X and Y chromosomes of *I. pacificus* and *I. scapularis* are significantly different in size (Oliver et al., 1993; Chen et al., 1994). We observed a wide variation in the genome size of the Ixodida, all of which were $1C \geqslant 1000$ Mbp with a mean of $1C = 1.31 \pm 0.07$ pg (~1.28 Gbp) for the Argasidae and $1C = 2.73 \pm 0.04$ pg (~2.67 Gbp) for the Ixodidae. The Ixodidae have larger genomes than Argasidae and the metastriate ticks have larger genomes than the prostriates. *O. turicata* had the smallest haploid genome size at approximately 1C = 1.12 pg (1.09±0.12 Gbp) while *A. americanum* had the largest at approximately 1C = 3.18 pg (3.12±0.03 Gbp).

The haploid genome size of the various tick species analyzed in this study is shown in comparison to those of a

Table 3
Comparisons in genome size between species, strains, and sexes within the Ixodida

Comparison groups	*p*-value	*t*-value	df	Significant difference
Within Genera				
Ixodes pacificus ♀ v. *Ixodes scapularis* ♀	0.0001	4.9	18	Yes
Ixodes pacificus ♂ v. *Ixodes scapularis* ♂	0.0001	6.79	18	Yes
Strains of same species				
Dermacentor andersoni ♀ Montana v. ID	0.25	1.2	14	No
Dermacentor andersoni ♀ Montana v. LG6OSU	0.32	1.04	12	No
Dermacentor andersoni ♀ ID v. LG6OSU	0.0036	4.62	6	Yes
Dermacentor andersoni ♂ Montana v. ID	0.0047	3.35	14	Yes
Dermacentor andersoni ♂ Montana v. LG6OSU	0.36	0.936	16	No
Dermacentor andersoni ♂ ID v. LG6OSU	0.0002	5.68	10	Yes
Between sexes				
Amblyomma americanum ♀ v. ♂	0.0001	12.8	19	Yes
Amblyomma cajennense ♀ v. ♂	0.0001	6.04	19	Yes
Amblyomma maculatum ♀ v. ♂	0.0001	8.95	22	Yes
Dermacentor andersoni Montana strain ♀ v. ♂	0.0009	3.9	20	Yes
Dermacentor andersoni ID strain ♀ v. ♂	0.0019	4.53	8	Yes
Dermacentor andersoni LG6OSU strain ♀ v. ♂	0.0075	3.55	8	Yes
Dermacentor variabilis ♀ v. ♂	0.12	1.6	20	No
Ixodes pacificus ♀ v. ♂	0.55	0.631	6	No
Ixodes scapularis ♀ v. ♂	0.0039	3.12	30	Yes

Comparison of average diploid (2C) genome size between species within genera, between male and female ticks of the same species and between strains of the same species. Statistical analyses were performed using a standard Student's *t*-test. Degrees of freedom (df), *p*- and *t*-values are provided. A *p*-value of $\leqslant 0.01$ was considered significant.

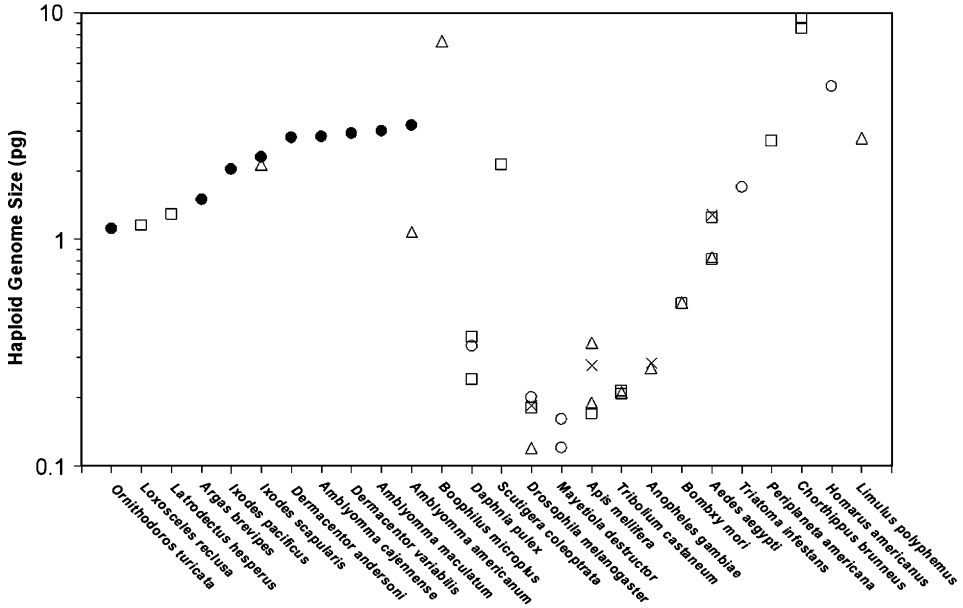

Fig. 2. Genome size of species of Arthropoda. Haploid genome size (GS) is shown in picograms (pg) on a logarithmic scale. Symbols indicate the method used to estimate genome size as follows: ○, flow cytometry; □, Feulgen densitometry; △, reassociation kinetics; X, whole genome shotgun sequencing. Open symbols represent genome sizes obtained from the Animal Genome Size Database (http://www.genomesize.com/) and the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genomes/static/gpstat.html) or as reported by Palmer et al. (1994) and Ullmann et al. (2005). Filled symbols represent genome sizes reported in this study. The common names of species listed are as follows: *A. aegypti* (yellow fever mosquito), *A. americanum* (lone star tick), *A. cajennense* (Cayenne tick), *A. maculatum* (Gulf Coast tick), *A. gambiae* (malaria mosquito), *Apis mellifera* (honey bee), *Bombyx mori* (silkworm moth), *B. microplus* (southern cattle tick), *C. brunneus* (field grasshopper), *D. pulex* (water flea), *D. andersoni* (Rocky Mountain wood tick), *D. variabilis* (American dog tick), *D. melanogaster* (fruit fly), *I. pacificus* (western black-legged tick), *I. scapularis* (black-legged tick), *H. americanus* (American lobster), *Latrodectus hesperus* (western black widow), *Limulus polyphemus* (horseshoe crab), *Loxosceles reclusa* (brown recluse spider), *M. destructor* (hessian fly), *O. turicata* (relapsing fever tick), *P. americana* (American cockroach), *Scutigera coleoptrata* (house centipede), *Triatoma infestans* (chagas kissing bug), and *Tribolium castaneum* (red flour beetle).

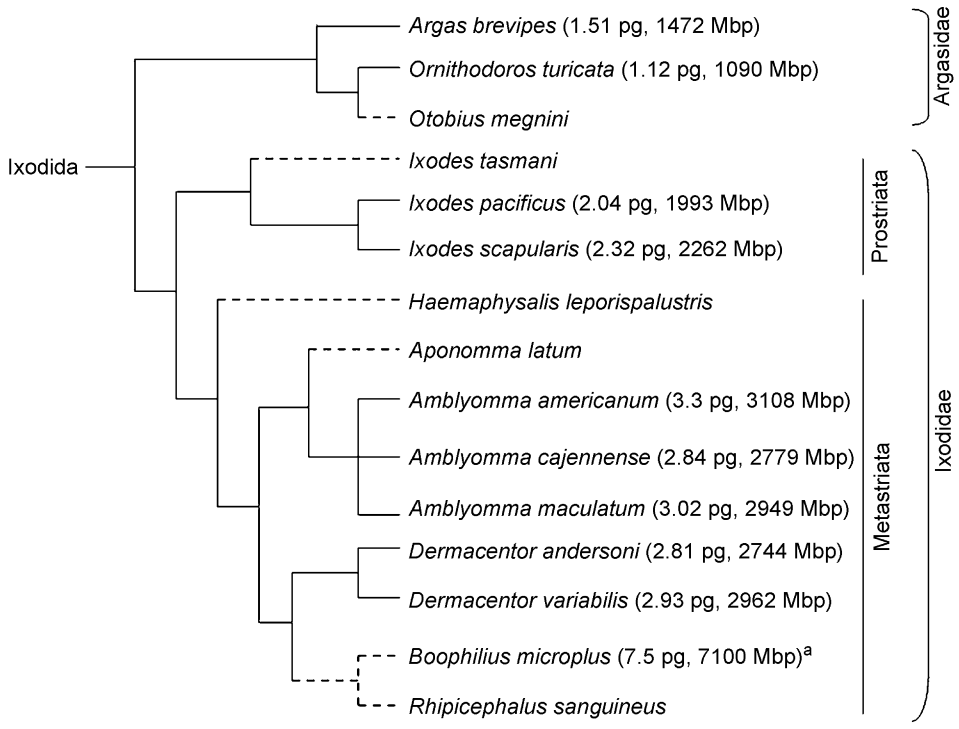

Fig. 3. Proposed phylogeny of the suborder Ixodida showing relationships between the families Ixodidae (hard ticks) and Argasidae (soft ticks) and the pro- and metastriate lineages of the Ixodidae and average haploid (1C) genome size in picograms (pg) and megabasepairs (Mbp) in parentheses. Filled lines indicate genome size estimations reported in this study. Phylogeny developed based on Balashov (1994), Klompen et al. (2000) and Barker and Murrell (2004) and personal communications with H. Klompen. Haploid genome size for species of Argasidae likely represents an average of measurements for males and females. [a]Genome size as reported by Ullmann et al. (2005). pg, picograms; Mbp, megabasepairs.

variety of arthropod species in Fig. 2. Genome size estimates not reported in this study were obtained from the Animal Genome Size Database (http://www.genomesize.com/), as determined by whole genome shotgun sequencing efforts (http://www.ncbi.nlm.nih.gov/genomes/static/gpstat.html), and as reported by Palmer et al. (1994) and Ullmann et al. (2005). The genome sizes of the Ixodidae and Argasidae fall within the range of known arthropod genome sizes but are larger than most arthropod genomes reported to date.

We used a Student's *t*-test to test for significant differences in diploid genome size between species of *Ixodes* and between strains and sexes of *D. andersoni* originating from different geographic regions in the western USA (James et al., 2006) (Table 3). A small but significant difference in genome size was determined between *I. scapularis* and *I. pacificus*. We observed a significant difference in genome size between *D. andersoni* female ID and LG6OSU strains, between males of the ID and Montana strains and between males of the ID and LG6OSU strains (Table 2). Significant differences between the genome sizes of male and female *A. americanum, A. cajennense, A. maculatum, D. andersoni* Montana, ID or LG6OSU strains and *I. scapularis* were observed but not between male and female *D. variabilis* and *I. pacificus*.

We examined genome size across the suborder Ixodida using a phylogeny developed based on the work of Balashov (1972), Klompen et al. (2000) (H. Klompen pers. commun) and Barker and Murrell (2004) (Fig. 3). We observed a trend towards larger genomes in species of ticks that are thought to be more recently diverged.

4. Discussion

Worldwide there are more than 899 species of ticks (suborder Ixodida) distributed in three families: the Argasidae (soft ticks), Ixodidae (hard ticks) and the Nuttalliellidae (comprising a single species which has not been collected for many years) (Sonenshine, 1991; Barker and Murrell, 2004). The majority of ticks are ectoparasites on wildlife but *ca.* 50 species are important pests of humans, livestock and companion animals. This was the first study to determine the genome size of a variety of hard and soft tick species of medical and veterinary significance using flow cytometry analysis. The study substantially extends knowledge of genome size across the suborder Ixodida and the phylum Arthropoda. Our findings inform on tick genome evolution and have important implications for genome research.

The haploid genome sizes of animals are known to vary more than 3000-fold from 0.04 pg in the placozoan *Trichoplax adhaerens* to over 130 pg in the marbled lungfish *Protopterus aethiopicus* (Gregory, 2001). The DNA content of many arthropod species has been determined using a variety of methods, revealing considerable variation in genome size across the phylum (Fig. 2). For example, the fruit fly *Drosophila melanogaster* and the water flea

Daphnia pulex have relatively small genomes of approximately 0.18 pg (176 Mbp) and 0.24 pg (235 Mbp), respectively, while certain species such as *Homarus americanus* (American lobster) and *Chorithippus brunneus* (field grasshopper) have some of the largest invertebrate genomes of 10.15 pg (9.93 Gbp) and 4.75 pg (4.65 Gbp), respectively. The argasid and ixodid ticks examined in this study have haploid genomes in excess of 1 pg (~1 Gbp) with a mean of 2.42 ± 0.04 pg (~2.36 Gbp). The mean haploid genome size of the Argasidae was 1.31 ± 0.07 pg (~1.28 Gbp) and that of the Ixodidae was 2.73 ± 0.04 pg (~2.67 Gbp). Our estimates of genome size for the Ixodida fall within the range determined thus far for species of Arthropoda and are relatively large in comparison to those of other arthropod species determined to date. The finding that *I. scapularis* and *B. microplus* repetitive DNA is organized in a pattern of short-period interspersion also supports this idea as short-period interspersion is typical of most animals with large genomes (Ullmann et al., 2005). However, it should be noted that the current invertebrate genome size data set is relatively small. As a consequence, surprisingly little is known of genome size variation in many invertebrate groups, especially the Ixodida. Furthermore, additional studies could reveal a wide range in genome size across the suborder Ixodida as has been noted for the mosquitoes (Rao and Rai, 1987). It is unlikely that large genomes are a characteristic unique to the Chelicerata, the Arachnida or even the Acari (mites and ticks). An extensive study of over 115 species of spiders in the infraorder Araneomorphae revealed genome sizes between 0.74 pg and 5.73 pg in this group of Arachnids (Gregory and Shorthouse, 2003) and unpublished studies using fluorescent densitometry suggest that at approximately 75 Mbp or 0.08 pg, the two-spotted spider mite has one of the smallest invertebrate genomes analyzed to date (Dearden et al., 2002). Studies using many invertebrate species are essential in order to identify trends, if any, in genome size across the phylum.

The phylogeny of the Ixodida has been reviewed in a number of studies (Balashov, 1994; Klompen et al., 2000; Barker and Murrell, 2004). To investigate possible trends in tick genome size, we mapped genome size onto a proposed phylogeny of the suborder Ixodida developed based on the above-mentioned studies. Hard ticks are thought to have evolved from bird-feeding soft ticks similar to *Argas* (Black and Piesman, 1994; Ribeiro et al., 2006) and the Argasidae are considered more basal than the Ixodidae. The Ixodidae consists of two major subdivisions: the Prostriata comprises the single genus *Ixodes* and is considered more basal than the Metastriata (Oliver, 1982; Klompen et al., 2000) which comprises all other species of hard ticks. Species of Ixodidae investigated in this study were found to have larger genomes than the Argasidae. Within the Ixodidae, the metastriate species had larger genomes than the prostriate species. Taken together, our results suggest a general trend toward larger genome size in more recent species of the Ixodida. It is important to note that genome size can change, in either direction, by various processes that operate at many physical and temporal scales (Gregory, 2005b). Thus, these findings may reflect a gain of DNA in more derived metastriates and/or a loss by the more basal Argasidae.

Ribeiro et al. (2006) identified a significant number of *I. scapularis* salivary proteins that appear to have evolved from gene duplication events. It is well known that genome evolution is not dominated by significant changes in the characteristics of coding sequences (Gregory, 2004). While an increase in tick coding sequence would contribute to total DNA content, the most likely explanation for large genome size in the Ixodida is the presence of significant amounts of non-coding genomic DNA such as repeat elements, pseudogenes, intergenic sequence and introns. Correlation between genome size and repetitive elements has been widely reported (Uozu et al., 1997; SanMiguel et al., 1998; Vieira et al., 2002). Ullmann et al. (2005) reported that the *I. scapularis* and *B. microplus* genomes are distinct from those of other arthropods in that they contain a greater proportion of moderately repetitive DNA relative to highly repetitive and unique DNA. Moderately repetitive DNA includes transposable elements (TEs) and members of multiple copy gene families such as the ribosomal RNA cistron. TEs are mobile genetic elements capable of replicating and spreading through genomes and often represent a significant portion of eukaryotic genomes (Kidwell, 2002; Tu and Coates, 2004; Gregory, 2005b). Nearly 45% of the *Homo sapiens* genome is composed of TEs, including long-interspersed nuclear elements (LINEs), short-interspersed nuclear elements (SINEs), DNA transposons, and long terminal repeat (LTR) retrotransposons (International Human Genome Sequencing Consortium, 2001). R2 retrotransposons have been reported in the *I. scapularis* genome (Bunikis and Barbour, 2005) but the classes, copy number and distribution of TEs in tick genomes are largely unknown. The IGP will inform on the relative contributions of TEs, other repeat elements and non-coding sequence to tick genome size.

Gene duplication in *I. scaplaris* (Ribeiro et al., 2006) and an increase in genome size could be the direct result of segmental duplication or possibly ancient genome duplication events (paleopolyploidy). Ribeiro et al. (2006) suggest that early in tick evolution, genome duplications occurred once, perhaps twice, from the ancestral mite that gave rise to the superorder Parasitiformes. Our finding of a greater than two-fold increase in average genome size between the Argasidae and Ixodidae and the published *B. microplus* genome size (Ullmann et al., 2005) supports the idea of one or multiple duplication events in the Ixodida. Cytogenetic studies are thus essential to investigate the role of duplication and perhaps even polyploidy in tick genome evolution.

Significant variation in genome size between species within the same genus has been documented. The tsetse fly vectors of African Trypanosomiasis (sleeping sickness), *Glossina morsitans* and *Glossina palpalis palpalis* have

genome sizes of approximately 0.61 pg (600 Mbp) and 7.16 pg (7000 Mbp), respectively (Aksoy et al., 2005). We compared the genome size of two prostriate ticks, *I. scapularis* and *I. pacificus* (Table 3). *I. pacificus* is endemic to the western coast while *I. scapularis* is endemic to the northeast, southeast, and Great Lakes regions of the US Both species are competent vectors of the Lyme disease pathogen *Borrelia burgdorferi* but they exploit different primary hosts and occupy different off-host habitats. The molecular phylogenetic analysis of Xu et al. (2003) places *I. scapularis* and *I. pacificus* in separate clades of the paraphyletic *I. ricinus* complex with *I. scapularis* the more basal of the two species. It has been proposed that the acquisition of vector competence for the *Borrelia* spirochete may have multiple origins in the *I. ricinus* complex (Xu et al., 2003). We observed a statistically significant difference in genome size between *I. scapularis* and *I. pacificus*, suggesting that substantial changes have arisen in the genomes of these two vectors since their divergence. The *I. scapularis* genome sequence will likely be an invaluable resource for comparative analyses with *I. pacificus*. However, sequence analysis will be essential to determine the relationship between coding and non-coding DNA sequence and the biology of these two vectors.

We compared diploid genome size of three strains of *D. andersoni* to determine variation, if any, between strains of the same species (Table 2). Pair-wise comparisons revealed small but significant differences in genome size between females of the ID and LG6OSU strains, males of the ID and Montana strains and males of the ID and LG6OSU strains. Similar findings were reported by Gregory and Shorthouse (2003) using different populations of the spider *Argiope aurantia*. Balashov (1989, 1994) proposed that the *Dermacentor* lineage evolved in Afrotropical forest and subsequently dispersed to the Neartic from Eurasia through the Bering land bridge and from Europe via Greenland during the Oligocene (35 million years ago). The evolutionary relationships and distance between the ID, Montana and LG6OSU strains are unknown, but our study suggests their genomes have accrued small and in some cases, significant changes since their divergence. Comparative genomics approaches between species and strains will likely be a powerful tool to investigate the biology of the Ixodida.

Differences in the diploid genome size of male and female tick species provide an indication of variation in DNA content between the sexes (Table 1). The diploid female genome of *A. americanum* is approximately 1 pg (978 Mbp) larger than that of the male. This result suggests that the X chromosome of this species may be composed of as much as 1 pg of DNA because *A. americanum* has an XX:X0 sex determination system. This idea is further supported by the karyotype analysis of Gunn and Hilburn (1995) who reported a large X chromosome relative to the autosomes in *A. americanum, A. cajennense,* and *A. maculatum*. We found no significant difference in genome size between female and male *I. pacificus* and *I. scapularis* suggesting that the X and Y chromosomes of these species may be similar in size or contain similar amounts of DNA. However, Chen et al. (1994) observed the Y chromosomes to be shorter than the shortest autosomes and the X chromosome to be the longest of all chromosomes in *I. scapularis* cell lines. As noted above, genome and chromosome size do not necessarily correlate. Assuming that the chromosomes of highly derived cell lines are truly representative of the karyotype, it may be that the Y chromosome of *I. scapularis* is significantly more condensed than the X.

In general, the prostriate ticks have XX:XY sex determination systems while the metastriates have an XX:XO system. The sex determination system of *A. brevipes* and *O. turicata* is unknown, but is expected to be XX:XY, based on that of other argasid species analyzed by Oliver (1982). Oliver (1982) proposed that the Y chromosome may have been lost as the metastriates emerged from earlier ancestors and the 2*n* number of chromosomes was reduced. Such events could provide a mechanism for genome size reduction. However, our results suggest a correlation between the evolution of an XX:XO system and an increase in genome size of the Ixodida. Species with an XX:XY sex determination system (*Argas*, *Ornithodoros* and *Ixodes* spp.) analyzed in this study have smaller genomes than the metastriate species. Other studies have failed to find an association between genome size and chromosome size or number (Gregory 2005b). Many forces likely operate to shape tick genome evolution and at present, the relationship between tick sex determination and genome size, if any, remains unclear.

Our 2.31 pg estimate of the *I. scapularis* haploid genome compares favorably with that of Ullmann et al. (2005). Minor differences between the two estimates could be due to the fact that our analysis was performed using female samples only while that of Ullmann et al. (2005) used egg batches composed of male and female ticks. Our haploid estimate for *A. americanum* females is 3.18 pg or approximately three times the 1.08 pg estimate of Palmer et al. (1994). The reasons for this difference remain unclear. We used an *A. americanum* strain which was established from the colony used by Palmer et al. (1994); it is unlikely that this colony, which has been in continuous culture since establishment, has undergone such a significant change in genome composition in the 12 years since the Palmer study. The difference in *C* values is most likely attributable to differences between flow cytometry and reassociation kinetics, especially as reassociation kinetics may underestimate genome size (Bennett et al., 2003; Schmuths et al., 2003; Dolezel and Bartos, 2005).

Our study has important implications for the IGP and tick genomics in general. An understanding of genome size and composition is essential for genome sequencing projects. With a haploid genome of approximately 2.3 Gbp, *I. scapularis* will be the largest arthropod genome

sequencing project undertaken to date. The size of the genome will necessitate significant shotgun sequencing in order to achieve a level of coverage sufficient for genome assembly and annotation. *I. scapularis* genome composition may also present challenges for genome assembly because regions of the genome with low sequence complexity may be difficult to assemble. However, tick genomic sequencing will provide much needed information on the nature of coding and non-coding sequence, the organization of these sequences in the genome, and their contribution to genome size. Genome sequence from *I. scapularis* and other ixodid ticks will provide an unprecedented opportunity to learn about the nature, organization and evolution of large genomes in the phylum Arthropoda.

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