University of Alberta

Genetic diversity and host specificity in the winter tick - *Dermacentor albipictus* (Acari: Ixodidae)

by

Sarah Sin Tong Leo

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Systematics and Evolution

Department of Biological Sciences

Fall 2012
Edmonton, Alberta

For my parents
Tsorng Ren Leo & Hung Khim Kua
And my little brother
Shaun Leo

♥ Thank You ♥

ABSTRACT

Dermacentor albipictus (Packard) is an obligate, hematophagous ectoparasite commonly found on large ungulates in North America. At high infestation levels, the winter tick is known to cause severe pathology and may transmit diseases to its hosts. Knowledge of the genetic diversity in this parasite will allow us to accurately identify the tick to species and better understand how it interacts with its hosts and surroundings. In this thesis, I developed and used 14 microsatellite loci to re-examine the species boundaries and to investigate host specificity in this tick. I confirmed that *D. albipictus* consists of a single species but exhibits extensive genetic variation that is more associated with geography than host species. Information on species boundaries, geographically-associated genetic variation and extent of host specificity in winter ticks can have important implications in pest control and further research is desirable.

ACKNOWLEDGEMENTS

There are many people I would like to thank for their help and generous support over the years. First on the list is my supervisor, Dr. Felix Sperling, for his unending patience and invaluable advice (on both my thesis work and regarding life in general). I am extremely fortunate that he was willing to take me under his wing as a summer undergraduate research student in 2007. I would also like to thank the members of my supervisory committee, Dr. Andrew Keddie, Dr. Kinga Kowalewska-Grochowska, and Dr. Margo Pybus, for their guidance and assistance. I have learnt much from them and they have each contributed to my thesis, be it in the form of specimen collection or valuable advice.

I am extremely thankful to my family for their unwavering support, unconditional love and unlimited understanding, particularly when stress made me snappish and horrible company to be around, much less live under a same roof with.

I am grateful to all members of the Sperling lab (both past and present) who have been most generous in sharing their experiences and wisdom. Heather Bird, Sean Bromilow, Bryan Brunet, Marie Djernaes, Jason Dombroskie, Julian Dupuis, Giovanny Fagua Gonzalez, Monica Higuera, Christi Jaeger, Patrick James, Jasmine Janes, Lisa Lumley, Benjamin (Ben) Proshek, Adrianne Rice, Marla Schwarzfeld, and Thomas Simonsen. Thank you for all the laughter and simulating (if sometimes disturbing) conversations over lunch breaks and for always being available when I needed to rant or pick your brains. Special thanks go to those who were invaluable sources of information and support when dealing with analysis programs: Dr. Corey Davis, Dr. William Samuel, Aaron Schafer, and Alexander Smith.

Last but not least, I would like to express my sincerest gratitude to the people and organisations who had been so wonderful and dependable when it came to gathering specimens for my project: Alberta Fish and Wildlife (Dr. M. Pybus and crew), City of Edmonton Environmental Services Lab, Elk Island

National Park (N. Cool and crew), Yukon Department of Environment, Isle Royale National Park, Minnesota Department of Natural Resources, Wyoming Game and Fish Department, United States Department of Agriculture (New Hampshire – Dr. A. Musante), Idaho Department of Fish and Game (Dr. M. Drew and crew), Alberta Agriculture and Rural Development (R. Cassis), Beck Veterinary Clinic Ltd., G. Anweiler, S. Cole, S. Curtis, S. Drew, C. Kashivakura, A. Keddie, S. Klatt, G. Lawrence, G. LeDrew, K, Moran, S. Nocholson, P. Pelly, G. Pohl, H. Proctor, C. Sabins, K. Shufran, J. Sperling, the Vankosky family, L. Veldkamp, J. Wilson, T. Zaedow (and any others that have remained nameless or we may have missed). Without your help I would never have been able to obtain all these specimens. Your contributions were fundamental to the completion of this project.

This study was funded by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to Dr. Felix Sperling.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

MYA: million years ago

COI: cytochrome oxidase I

16SrRNA: 16S ribosomal RNA

ITS-2: internal transcribed spacer 2

PCR: polymerase chain reaction

mtDNA: mitochondrial DNA

MCMC: Markov Chain Monte Carlo

EST: expressed sequence tags

HWE: Hardy-Weinberg equilibrium

FLE: *Francisella*-like endosymbiont

MP: maximum parsimony

ML: maximum likelihood

CHAPTER ONE

General Introduction

Ticks

Ticks (Order: Ixodida) are members of the phylum Arthropoda. They belong in the class Arachnida and form the sub-class Acari with mites. There are currently three recognised families of ticks: the Ixodidae (hard ticks, 702 species), the Argasidae (soft ticks, 193 species), and the Nuttalliellidae (monotypic, *Nuttalliella namaqua* in South Africa) (Keirans *et al*. 1976, Barker and Murrell 2004, Guglielmone *et al.* 2010, Mans *et al.* 2011). Ticks are presumed to be early lineages of terrestrial arachnids and are proposed to have originated between the late Silurian and the late Cretaceous (443 – 65 million years ago (MYA)) (Hoogstraal and Aeschlimann 1982, Lindquist 1984, Oliver 1989, Balashov 1994, Klompen *et al.* 1996, de la Fuente 2003). The oldest tick fossil found to date is a soft tick preserved in amber approximately 90 – 94 million years old (Klompen and Grimaldi 2001), suggesting that ticks might have already radiated into the families we see today during the Cretaceous period.

Ticks are obligate, hematophageous ectoparasites, recognised as important veterinary and medical threats second in importance only to mosquitoes (Spach *et al.* 1993, Allan 2001, Parola and Raoult 2001). They have been found feeding on a wide variety of organisms including birds, mammals, reptiles, and amphibians (Bishopp and Trembley 1945, Oliver 1989, Burridge 2001, Smith *et al.* 2008). Blood-feeding behaviour in ticks is believed to have evolved in an ancestral tick lineage, with the different mechanisms for hematophagy evolving through multiple independent events between 92 – 120 MYA (Mans *et al.* 2002, Mans and Neitz 2004, Mans *et al.* 2011).

Ticks are known for their ability to induce severe pathology (*e.g.* tick paralysis, anemia, and severe allergic reactions) in their hosts (Bishopp and Trembley 1945) and can vector a number of diseases including Lyme disease,

tularaemia, ehrlichiosis, and anaplasmosis (Bratton and Corey 2005). With midguts that are uniquely suitable for pathogen survival and long feeding periods interspersed with periods of ingestion and regurgitation, ticks are well adapted for effective disease transmission (Parola and Raoult 2001). Ticks can also act as reservoirs of tick-borne diseases by maintaining pathogens in a population via transstadial (between life stages) and transovarial (from female to offsprings) transmission (Parola and Raoult 2001).

As important medical and veterinary pests, it is critical that we be able to identify tick species accurately and increase our understanding of interactions between ticks and their surroundings. Such knowledge will be invaluable for implementing efficient monitoring and control programs. Information on parasite genetic diversity can potentially serve as a tool for accurate identification of pest species and for increasing our understanding of host-parasite-pathogen interactions (Stockwell and Leberg 2002, Armstrong and Ball 2005, Magalhães *et al.* 2007). In this thesis, I focus on the genetic diversity of a particular species of hard tick that can be found throughout North America- *Dermacentor albipictus*, also known as the winter tick or moose tick.

The winter tick – *Dermacentor albipictus*

Dermacentor albipictus (Packard 1896) is an obligate ectoparasite that commonly parasitises large ungulates like elk (*Cervus elaphus canadensis* (Erxleben)), moose (*Alces alces* (Linnaeus)), and horses (*Equus ferus caballus* Linnaeus). It can be found across North America (Bishopp and Trembley 1945, Yunker *et al*. 1986, Samuel 2004) (Figure 1-1, Appendix A). The winter tick can be various shades of brown with grey dorsal patterning (Figure 1-2). Some individuals that are well ornamented may exhibit iridescence (Cooley 1938). Like all Ixodidae, the winter tick has an oval body shape that narrows anteriorly with eleven festoons on the posterior end of the opisthosoma (Figure 1-2) (Cooley 1938). Its basis capitulum is rectangular when viewed both dorsally and ventrally

and is always wider than long (Figure 1-2A, 1-2C). Its palpi are short and broad. The hypostome is covered in three rows of dentition on either side of the median. Coxae I to IV increase in size posteriorly and spurs are present on all coxae (Figure 1-2B, 1-2D). Spiracle plates on *D. albipictus* are variable in shape but always consist of large goblet cells (Yunker *et al.* 1986, Leo *et al.* 2010). Dorsal prolongations on the spiracle plates may or may not be present (see Leo *et al.* 2010 Figure 1).

Life History

In Alberta, *Dermacentor albipictus* produces one generation per year. Six-legged larval ticks hatch around August and September and scale vegetation to begin questing for hosts (McPherson *et al*. 2000, Aalangdong and Samuel 2001, Allan 2001). Once on a host, the larvae immediately initiate feeding before molting into the eight-legged nymphal stage. Nymphs remain on the host throughout fall and winter, feeding continuously and becoming engorged. Once fully fed, the nymphs undergo their final molt to become sexually mature adults (January – May). Around March and April, mated females become engorged and detach from the host. They lay their eggs on the ground before expiring (Samuel 2004). Reproductive success of winter ticks is dependent on a variety of factors such as the host species parasitised, host avoidance and grooming behaviour, and weather conditions (Drew and Samuel 1987, Samuel and Welch 1991, Welch *et al.* 1991).

Importance in management and conservation

Heavy infestations of winter ticks on a host individual can result in severe anaemia, extensive epithelial damage, allergic reactions, loss of winter coats by hosts due to excessive grooming behaviour, and even death (McLaughlin and Addison 1986, Glines and Samuel 1989, Anderson 2002,). *D. albipictus* can also carry a variety of pathogens (Baldridge *et al*. 2009). It is considered an important veterinary and economical pest in the United States cattle industry due to its ability to transmit the bacteria *Anaplasma marginale* (Theiler) (Sonenshine 2003).

It is pertinent that we be able to identify *D. albipictus* to species at all life stages. Unfortunately, identifying ticks to species based on morphology is often difficult due to overlapping morphological traits (Brinton *et al.* 1965, Dergousoff and Chilton 2007). However, with recent advances in sequencing techniques, we are presented with an efficient method for species delimitation and identification. Although this method has many limitations and must be examined further for reliability, it can be very useful for pest species identification (Armstrong and Ball 2005, Rubinoff *et al.* 2006). This is particularly so when dealing with specimens of poor quality or juvenile stages (Hebert *et al.* 2003).

Host-parasite interaction is a form of co-evolution whereby parasites and hosts reciprocally respond to selection pressures imposed by each other (Clayton *et al.* 1999). To counter parasite-induced pathology, host animals invest resources in costly immune defences (Sheldon and Verhulst 1996). In response, parasites evolve specialised mechanisms to avoid or overcome host immune defences (Antia and Lipsitch 1997, Gillespie *et al*. 2000). This evolutionary arms race between parasites and their hosts is an example of the "Red Queen Hypothesis" (Van Valen 1973). It is important that we obtain a better understanding of host-parasite relationships as these interactions can affect host fitness in the form of decreased reproductive success (Baudoin 1975, Ebert and Herre 1996) and death (Boots and Sasaki 2002, 2003). The ability to associate genetic 'strains' of *D. albipictus* ticks to particular host species could be potentially useful for risk assessment of tick populations in a region.

Microsatellites

In recent years, it has become increasingly easier for researchers to carry out fine-scaled genetic studies using markers like single-nucleotide polymorphisms (SNPs) and short tandem repeat regions such as microsatellites. Microsatellites are regions of the genome that usually consist of repeating regions 1 to 6 nucleotides in length (Li *et al.* 2002, Kelkar *et al*. 2010). As co-dominant and (presumably) selectively neutral markers, microsatellites are ideal markers for

studies that investigate population structures, pedigree, kinship, and in some cases, species boundaries (Blouin *et al.* 1996, Kelkar *et al.* 2010, Lumley and Sperling 2011,). An added bonus to using microsatellites markers is that the evolutionary mechanism behind these markers is reasonably well understood (Schlötterer 2000).

To date, most molecular studies on *D. albipictus* have been done via sequencing of common mitochondrial (16S ribosomal RNA and cytochrome oxidase I) and nuclear (internal transcribed spacer 1 and 2, lysozyme) markers (Crosbie *et al.* 1998, Leo *et al.* 2010). Using additional markers such as microsatellites can allow us to obtain better understanding of winter tick species boundaries and genetic variability.

Scope of thesis

The goal of my M.Sc. thesis is three-fold. My first objective is to develop a set of microsatellite markers that can be used as additional tools for studying *D. albipictus* genetic diversity in North America (Chapter 2). My second goal is to re-examine the species boundaries of *D. albipictus* through extensive genomic and geographical sampling (Chapter 3). Finally, I investigate the degree of host preference in *D. albipictus* whose host-dependent life cycle and limited dispersal capabilities may contribute selective pressures that can result in host specialisation (Chapter 4).

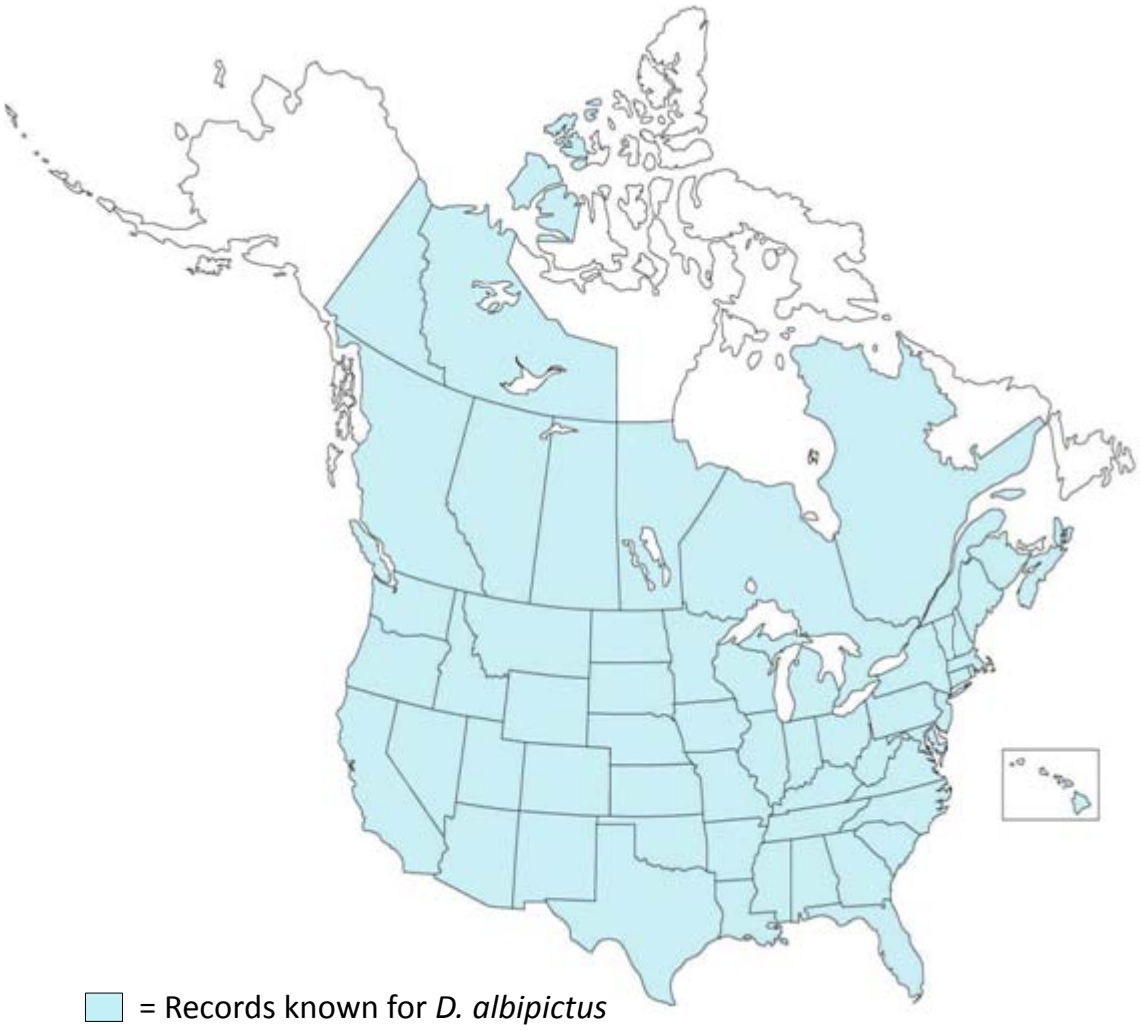


Figure 1-1. Map indicating the approximate jurisdictional distribution of *Dermacentor albipictus* ticks in Canada and the United States of America. States and provinces from which *D. albipictus* has been reported are coloured. Distribution is established based on literature reporting where *D. albipictus* has been collected or is present. Winter ticks are not reported in Nunavut, Newfoundland and Labrador, Prince Edward Island, Delaware and Alaska. A complete list of the sources used to create this distribution map is listed in Appendix A.

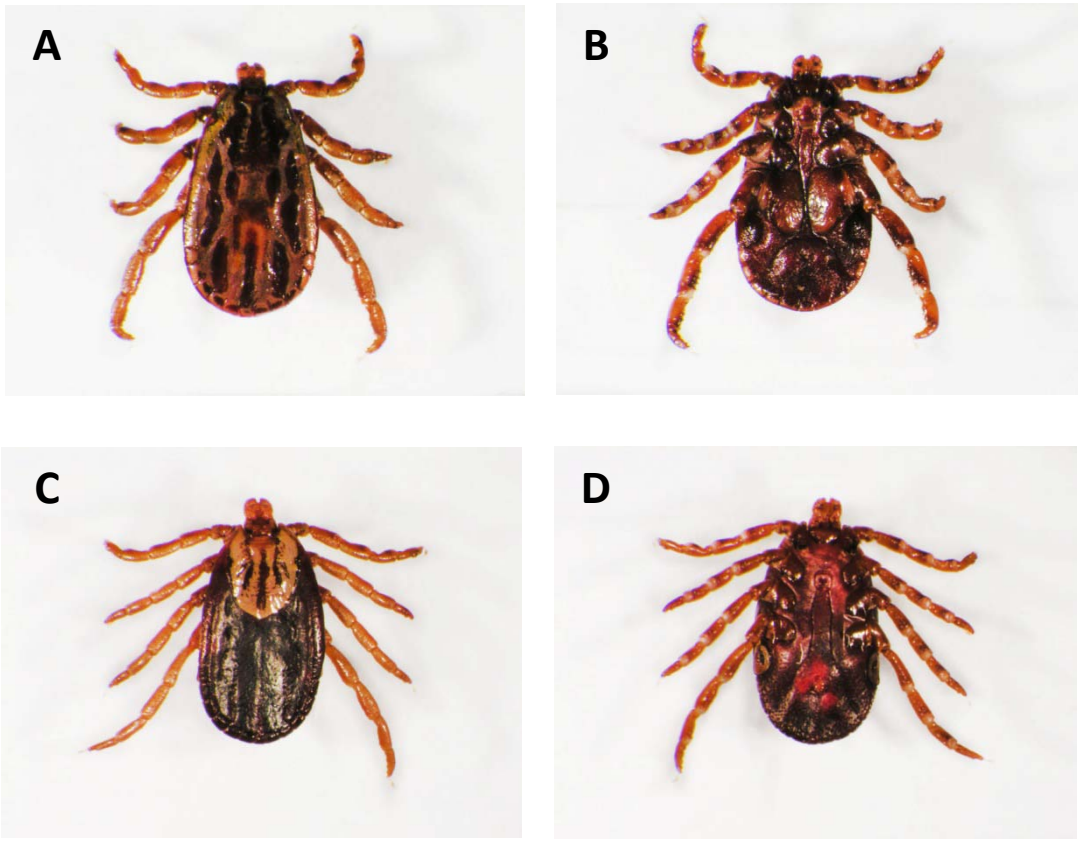


Figure 1-2. Images of adult *Dermacentor albipictus* ticks: (A) dorsal view of a male, (B) ventral view of a male, (C) dorsal view of a female, (D) ventral view of a female. These images are available on the University of Alberta Strickland Virtual Museum (http://entomology.museums.ualberta.ca/searching_species_details.php?s=31506).

Literature Cited

Aalangdong OI, Samuel WM. 2001. Questing behaviour and orientation of larval winter ticks, *Dermacentor albipictus* (Packard) (Acari: Ixodidae) on vegetation in Central Alberta, Canada." *J Ghana Sci Assoc* **3:** 93-98.

Allan SA. 2001. Ticks (Class Arachnida: Order Acarina). In: Parasitic diseases of wild mammals 2nd Edition (*eds* Samuel WM, Pybus MJ, Kocan AA). Iowa State University Press, Iowa, pp. 72 – 106.

Anderson JF. 2002. The natural history of ticks. *Med Clin North Am* **86**: 205- 218.

Antia R, Lipsitch M. 1997. Mathematical models of parasite responses to host immune defences. *Parasitol* **115**: S155 - S167.

Armstrong KF, Ball SL. 2005. DNA barcodes for biosecurity: Invasive species identification. *Phil Trans R Soc B* **360**: 1813-1823.

Balashov YS. 1994. Importance of continental drift in the distribution and evolution of ixodid ticks. *Entomol Rev* **73**: 42 – 50.

Baldridge GD, Scoles GA, Burkhardt NY, Schloeder B, Kurtti TJ, Munderloh UG. 2009. Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma* phagocytophilum variants in *Dermacentor albipictus* (Acari: Ixodidae). *J Med Entomol* **46**: 625 – 632.

Barker SC, Murrell A. 2004. Systematics and evolution of ticks with a list of valid genus and species names. *Parasitology* **129**: S15–S36.

Baudoin M. 1975. Host castration as a parasitic strategy. *Evolution* **29**: 335-352.

Bishopp FC, Trembley HL. 1945. Distribution and hosts of certain North American ticks. *J Parasitol* **31**: 1-54.

Blouin MS, Parsons M, Lacaille V, Lotz S. 1996. Use of microsatellite loci to classify individuals by relatedness. *Mol Ecol* **5**: 393–401.

Boots M, Sasaki A. 2002. Parasite-driven extinction in spatially explicit host-parasite systems. *American Naturalist* **159**: 706-713.

Boots M, Sasaki A. 2003. Parasite evolution and extinctions. *Ecology Letters* **6**: 176-182.

Bratton RL, Corey GR. 2005. Tick-borne disease. *Am Fam Phys* **71**: 2323-2330.

Brinton EP, Beck DE, Allred DM. 1965 Identification of the adults, nymphs and larvae of ticks of the genus *Dermacentor* Koch (Ixodidae) in the Western United State. *Brigham Young Univ Sci Bull Biol Ser* **5**: 1-44.

Burridge MJ. 2001. Ticks (Acari: Ixodidae) spread by the international trade in reptiles and their potential roles in dissemination of diseases. *Bull Entomol Res* **91**: 3-23.

Clayton DH, Lee PL, Tompkins DM, Brodie ED III. 1999. Reciprocal natural selection on host parasite phenotypes. *American Naturalist* **154**: 261-270.

Cooley RA. 1938. The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation. *Nat Inst Health Bull* **171**: 59- 64.

Crosbie PR, Boyce WM, Rodwell TC. 1998. DNA sequence variation in *Dermacentor hunteri* and estimated phylogenies of *Dermacentor* spp. (Acari: Ixodidae) in the New World. *J Med Entomol* **35**: 277- 288.

de la Fuente J. 2003. The fossil record and the origin of ticks (Acari: Parasitiformes: Ixodida). *Exp Appl Acarol* **29**: 331 – 334.

Dergousoff SJ, Chilton NB. 2007. Differentiation of three species of Ixodid tick, *Dermacentor andersoni*, *D. variabilis* and *D. albipictus*, by PCR-based approaches using markers in ribosomal DNA. *Mol Cell Probes* **21**: 343- 348.

Drew ML, Samuel WM. 1987. Reproduction of the winter tick, *Dermacentor albipictus*, under laboratory conditions. *Can J Zool* **64**: 2583-2588.

Ebert D, Herre EA. 1996. The evolution of parasitic diseases. *Parasitol Today* **12**: 96-100.

Gillespie RD, Mbow ML, Titus RG. The immunomodulatory factors of bloodfeeding arthropod saliva. *Parasit Immunol* **22**: 319-331.

Glines MV, Samuel WM. 1989. Effect of *Dermacentor albipictus* (Acari: Ixodidae) on blood composition, weight gain and hair coat of moose, Alces alces. *Exp Appl Acarol* **6**: 197- 213.

Guglielmone AA, Robbins RG, Apanaskevich DA, Petney TN, Estrada-Peña A, Horak IG, Shao R, Barker SC. 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa* **2528**: 1-28.

Hebert PDN, Cywinska A, Ball S, deWaard JR. 2003. Biological identification through DNA barcodes. *Proc R Soc Lond B* **270**: 313-321.

Hoogstraal H, Aeschlimann A. 1982. Tick-host specificity. *Bulletin de la Société*

Entomologique Suisse **55**: 5–32.

Keirans JE, Clifford CM, Hoogstraal H, Easton ER. 1976. Discovery of *Nuttalliella namaqua* Bedford (Acarina: Ixodoidea: Nuttalliellidae) in Tanzania and redescription of the female based on scanning electron microscopy. *Ann Entomol Soc Amer* **69**: 926–932.

Kelkar YD, Strubczewaki N, Hile SE, Chiaromonte F, Eckert KA, Makova KD. 2010. What is a microsatellite: A computational and experimental definition based upon repeat mutational behaviour at A/T and GT/AC repeats. *Genome Biol Evol* **2**: 620-635.

Klompen JSH, Black WC, Keirans JE, Oliver JH. 1996. Evolution of ticks. *Ann Rev Entomol* **41**: 141–161.

Klompen H, Grimaldi D. 2001. First Mesozoic record of a parasitiform mite: A larval argasid tick in Cretaceous amber (Acari: Ixodida: Argasidae). *Ann Entomol Soc Am* **94**: 10–15.

Leo SST, Pybus MJ, Sperling FAH. 2010. Deep mitochondrial DNA lineage divergences within Alberta populations of *Dermacentor albipictus* (Acari: Ixodidae) do not indicate distinct species. *J Med Entomol* **47**: 565-574.

Li Y, Korol AB, Fahima T, Beiles A, Nevo E. 2002. Microsatellites: Genomic distribution, putative functions and mutational mechanisms: A review. *Mol Ecol* **11**: 2453 – 2465.

Lindquist EE. 1984. Current theories on the evolution of major groups of Acari and on their relationships with other groups of Arachnida with consequent implications for their classification. In: Acarology VI, vol. 1. (*eds* Griffiths DA, Bowman CE). John Wiley & Sons, New York, pp. 28–62.

Lumley LM, Sperling FAH. 2011. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). *Mol Phylogenet Evol* **58**: 232-243.

Magalhães S, Forbes MR, Skoracka A, Osakabe M, Chevillon C, McCoy KD. 2007. Host race formation in the Acari. *Exp Appl Acarol* **42**: 225-238.

Mans BJ, de Klerk D, Pienaar R, Latif AA. 2011. *Nuttalliella namaqua*: a living fossil and closest relative to the ancestral tick lineage: Implications for the evolution of blood-feeding in ticks. *PLoS ONE* **6**: e23675.

Mans BJ, Louw AI, Neitz AW. 2002. Evolution of hematophagy in ticks: Common origins for blood coagulation and platelet aggregation inhibitors from soft ticks of the genus *Ornithodoros*. *Mol Biol Evol* **19**: 1695–1705.

Mans BJ, Neitz AW. 2004. Adaptation of ticks to a blood-feeding environment: evolution from a functional perspective. *Insect Biochem Mol Biol* **34**: 1–17.

McLaughlin RF, Addison EM. 1986. Tick (*Dermacentor albipictus*)-induced winter hair-loss in captive moose (Alces alces). *J Wildl Dis* **22**: 502- 510.

McPherson M, Shostak AW, Samuel WM. 2000. Climbing simulated vegetation to heights of ungulate hosts by larvae of *Dermacentor albipictus* (Acari: Ixodidae). *J Med Entomol* **37**: 114 – 120.

Oliver JH. 1989. Biology and systematics of ticks (Acari: Ixodida). *Ann Rev Ecol Evol Sys* **20**: 397-430.

Parola P, Raoult D. 2001. Ticks and tickborne bacterial diseases in humans: An emerging infectious threat. *Cli Infect Dis* **32**: 897-928

Rubinoff D, Cameron S, Will K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *J Hered* **97**: 581-594.

Samuel WM. 2004. White as a Ghost: Winter Ticks and Moose. Federation of Alberta Naturalists, Edmonton, Alberta, Canada.

Samuel WM, Welch DA. 1991. Winter tick on moose and other ungulates: Factors influencing their population size. *Alces* **27**: 169-182.

Schlötterer C. 2000. Evolutionary dynamics of microsatellite DNA. *Chromosoma* **109**: 365-371.

Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol Evol* **11**: 317-321.

Smith RL, Schnack JA, Schaefer EF, Kehr AI. 2008. Ticks, *Amblyomma rotundatum* (Acari: Ixodidae), on toads *Chaunus schneideri* and *Chaunus granulosus* (Anura: Bufonidae) in northern Argentina. *J Parasitol* **94**: 560-562.

Sonenshine DE. 2003. Ticks. In: Encyclopedia of Insects. Elsevier Science, San Diego, California, USA, pp. 1132 – 1141.

Spach DH, Liles WC, Campbell GL, Quick RE, Anderson DE, Fritche TR. 1993. Tick-borne diseases in the United States. *N Engl J Med* **329**: 936- 947.

Stockwell CA, Leberg PL. 2002. Ecological genetics and the translocation of native fished: Emerging experimental approaches. *West N Am Natur* **62**: 32 – 38.

Van Valen L. 1973. A new evolutionary law. *Evol Theory* **1**: 1-30.

Welch DA, Samuel WM, Wilke CJ. 1991. Suitability of moose, elk, mule deer and white-tailed deer as hosts for winter ticks (*Dermacentor albipictus*). *Can J Zool* **69**: 2300-2305.

Yunker CE, Keirans JE, Clifford CM, Easton ER. 1986. *Dermacentor* ticks (Acari: Ixodoidea: Ixodidae) of the New World: A scanning electron microscope atlas. *Proc Entomol Soc Wash* **88**: 609-627.

CHAPTER TWO *

Isolation and characterization of 14 microsatellite loci developed for *Dermacentor albipictus* and cross-species amplification in *D. andersoni* and *D. variabilis* (Acari: Ixodidae)

*A slightly modified version of this chapter has been published:
Leo SST, Davis C, and Sperling FAH. 2011. *Conservation Genetics Resources*.
doi: 10.1007/s12686-011-9553-x

Winter ticks (*Dermacentor albipictus* (Packard)) are obligate ectoparasites that feed on blood of large ungulates. Heavy infestations of these parasites can result in severe pathology and host fatality (Samuel 2004). *D. albipictus* are also known vectors of *Anaplasma marginale* (Theiler) (Aubry and Geale 2011). To study *D. albipictus* biodiversity and population structure in North America, I developed microsatellite loci using three methods: (1) screening of genomic *D. albipictus* DNA via the universal linker and ligation protocol (Hamilton *et al.* 1999); (2) a genomic resource-based approach using expressed sequence tag (EST) libraries and *in silico* techniques; (3) adapting loci originally developed for *D. variabilis* (Dharmarajan *et al.* 2009).

Fourteen micrograms of genomic DNA was extracted from five *D. albipictus* specimens (collected from mule deer (*Odocoileus hemionus* (Rafinesque)) in Oyen, Alberta), fragmented with *NheI*, and enriched for dinucleotide repeats [$(GT)_{14}$ and $(CT)_{14}$] (Hamilton *et al.* 1999) using SuperSNX24 linkers from Glenn and Schable (2005). Enriched fragments were cloned into pBSII SK^+ and transformed into DH5α competent *Escherichia coli* cells. Insertion sequences of 259 recombinant clones were amplified using universal plasmid-specific T3 (5'AAT TAA CCC TCA CTA AAG GG3') and T7 (5'TAA TAC GAC TCA CTA TAG GG 3') primers in 50 µL reactions consisting of 1X PCR Buffer (50mM Tris-Cl, pH 9.2, 1.8mM $MgCl_2$, 10mM $(NH_4)_2(SO_4)$, 0.1mg/ml BSA, 0.0025% (v/v) β-mercaptoethanol), 0.2mM of each dNTPs, 25

pmol of each primer, 1U Taq, and 4µl template DNA. The PCR protocol consisted of 30 cycles of 94°C for 15s, 52°C for 30s, and 72°C for 45s, with a final extension at 72°C for 300s. Amplified products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA), sequenced using Big Dye Kit and protocol (Applied Biosystems, Foster City, CA), and visualised on an ABI3730 capillary sequencer (Applied Biosystems, Foster City, CA). Contigs of sequences were created using LaserGene (DNASTAR Inc, USA). Sixteen insertion sequences contained repeat motifs.

EST sequences for *D. variabilis* and *D. andersoni* were downloaded from the National Center for Biotechnology Information database and aligned using Lasergene (DNASTAR Inc, USA). Contigs were scanned for repeat regions using a PERL script designed to search for microsatellite repeats (Beldade *et al.* 2006). Twenty-four contigs exhibited at least six simple sequence repeats.

Primers were designed for the abovementioned 40 microsatellite loci using Primer3 (Rozen and Skaletsky 2000) and tested on *D. albipictus* specimens, along with 12 additional loci designed for *D. variabilis* (Dharmarajan *et al.* 2009). M13 tails were added onto the 5' end of all forward primers and amplification was performed using the protocol in Schuelke (2000). Microsatellite loci were amplified in 15 µL PCR cocktail consisting of 1X PCR Buffer (10mM Tris pH 8.8, 0.1% Triton X-100, 50mM KCl, 0.16mg/ml BSA), 2.4mM $MgCl_2$, 0.2 µM of each dNTPs, 4.8 pmol of the forward primer, 1.6 pmol reverse primer, 4.8 pmol labelled M13 primers, 1U Taq, and 4µl template DNA. Amplified products were diluted 1 in 5, co-loaded, and visualized on an ABI3730 automated capillary sequencer (Applied Biosystems, Foster City, CA) using GS500-LIZ (Applied Biosystems, Foster City, CA) as the size standard. Genbank accession numbers of sequences used to develop microsatellite loci are listed in Table 2-1.

Fourteen loci (4 from screening an enriched genome, 8 developed from the EST libraries, and 2 developed by Dharmarajan *et al.* (2009)) that amplified successfully were validated using a panel of 17 *D. albipictus* specimens (collected

during an elk (*Cervus elaphus canadensis* (Erxleben)) monitoring project in the Waterton region, Alberta) using labelled primers. *D. andersoni* and *D. variabilis* collected from British Columbia and Nova Scotia respectively were used in cross-species amplifications. Amplifications were performed in 15uL reactions containing 1X PCR buffer (10mM Tris pH8.8, 0.1% Triton X-100, 50mM KCl, 0.16mg/ml BSA), 3.5mM $MgCl_2$, 0.2mM each dNTPs, 2uM primers, 0.12U Taq and 2.5µl template DNA (extracted using the QIAamp DNA mini kit (Qiagen, Valencia, CA)). Amplification protocol included 35 cycles of 94°C for 15s, 59°C for 30s, and 72°C for 45s. Products were visualized on an ABI3730 capillary sequencer and genotyped using *GeneMapper*® with GS500-TAMRA as the size standard (Applied Biosystems, Foster City, CA).

Of the 14 loci that amplified successfully in three *Dermacentor* species, 11 were polymorphic in *D. albipictus* and *D. andersoni*, 12 in *D. variabilis* (Table 2-2). All loci were polymorphic in at least one species. The allele size ranges of each loci differed slightly with overlap between species. The number of alleles per locus ranged from 1 to 13 for *D. albipictus*, 1 to 15 for *D. andersoni* and 1 to 11 for *D. variabilis*, averaging at 4.07, 4.57, and 5.21, respectively. Loci developed from EST contigs tended to have fewer unique alleles and narrower allele size ranges compared to those developed from screening genomic DNA (Table 2-2). This is likely because ESTs are generally evolutionarily conserved regions of the genome and less prone to uncorrected slippage errors during DNA replication (Kelkar *et al.* 2010).

Relevant genetic diversity statistics for 11 polymorphic microsatellite loci in *D. albipictus* were obtained using Excel Microsatellite Toolkit (Park 2001) (Table 2-2). Expected and observed heterozygosity for polymorphic loci ranged from 0.369 to 0.889 (averaging 0.623) and 0.294 to 0.824 (averaging 0.518) respectively.

Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium tests were performed in Genepop 4.0.1 (Raymond and Rousset 1995, Rousset 2008).

Linkage disequilibrium tests revealed no significantly linked loci after applying Bonferroni correction. HWE analyses revealed significant heterozygote deficiencies in loci 6F and 3B after Bonferroni correction, but only locus 6F was significantly out of HWE. This may be due to the Wahlund effect, in which the presence of subpopulation structure may result in decreased heterozygosity. Compared to most other *Dermacentor* ticks *D. albipictus* has an uncommon one-host life cycle (Samuel 2004) that may increase chances of inbreeding. However, since most loci were not significantly out of HWE, this lack of conformity to HWE is more likely due to null alleles (whereby mutations on the primer annealing site result in unsuccessful amplification of the allele). MICROCHECKER (van Oosterhout *et al.* 2004) identified both loci 6F and 3B as potentially having null alleles due to excess homozygosity.

Although not all 14 microsatellite loci were polymorphic or in HWE, they are still potentially informative. Differences in size ranges between species are potentially useful in delimiting species boundaries (Lumley and Sperling 2011). Loci that are monomorphic in one species may be polymorphic in another. Furthermore, the validation panel in this study is a limited representation of the species in North America. Monomorphic loci in this panel may not remain so once genotyped across a wider geographical range (Jiang *et al.* 2011). Therefore, all 14 loci are useful markers and will be included in future studies on *D. albipictus* species boundaries and genetic diversity across North America (Leo *et al.* 2010).

Table 2-1. Genbank accession numbers of sequences used to develop the 14 microsatellite loci. Sources for all sequences are listed.

Microsatellite Loci	Genbank Accession Numbers	Source
3B	JF749832	Previously unpublished
6F	JF749835	Previously unpublished
3-5E	JF749834	Previously unpublished
162	JF749833	Previously unpublished
est-068	EX744344, EX744141	Anderson *et al.* 2008
est-102	EZ532493, EZ539996, EZ524700, EZ533776, EZ532354, EZ524833	Jaworski *et al.* 2010
est-103	EZ539968, EZ529487, EZ524744	Jaworski *et al.* 2010
est-120	EZ532141, EZ524558, EZ540066	Jaworski *et al.* 2010
daest-017	EG364140, EG363695, EG364259, EG363924, EG36429	Alarcon-Chaidez *et al.* 2007
daest-135A	EG363646, EG363364, EG363985	Alarcon-Chaidez *et al*. 2007
daest-135B	EG363646, EG363364, EG363985	Alarcon-Chaidez *et al*. 2007
daest-212	EG364090, EG363932, EG363408	Alarcon-Chaidez *et al*. 2007
DV-28	EF545257	Dharmarajan *et al*. 2009
DV-31	EF545250	Dharmarajan *et al*. 2009

Table 2-2. Characteristics and summary statistics of microsatellite loci genotyped in *Dermacentor albipictus*, *D. andersoni*, and *D. variabilis*. The forward primers are labelled with fluorescent dyes *6-FAM* (*F*), *TET* (*T*), and *HEX* (*H*). Number of individuals for which loci successfully amplified (*N*), number of alleles (N_a), observed and expected heterozygosity (H_O and H_E respectively). '*' indicates deviation from Hardy-Weinberg equilibrium (HWE) (p-value < 0.0036) after Bonferroni correction.

			D. albipictus				***D. andersoni***			***D. variabilis***		
Locus	**Primer Sequence (5' – 3')**	**Repeat motif**	***N***	***N_a***	***H_O (H_E)***	**Size Ranges**	***N***	***N_a***	**Size Ranges**	***N***	***N_a***	**Size Ranges**
3B	*F* - CGAAGCTTTCTCTGCCTAAACT ACCCATAGCCACTCAGCAAC	$(CA)_{15}$	17	9	0.500 (0.792)	149-175	7	6	139-159	9	6	139-165
6F	*T* - TCACTATAGGGCGAATTGGGTA CTAAAGGGAACAAAGCTGGAG	$(CA)_7$	17	13	0.294* (0.889)	137-205	17	15	137-259	15	11	145-225
5E	*H* - CACTTTCGTAACGAGGATTTGA AGGATACCAGATGGTCACTTGC	$(CAA)_{24}$	13	5	0.294 (0.369)	210-228	17	11	186-216	17	8	177-210
162 [a]	*T* - CCCAACACCATAGCCACT G AAAAATCGTCGCAGTTTTGC	$(GT)_{40}$	13	4	0.375 (0.742)	353-356	10	7	330-370	6	6	306-372
est-068	*T* -AAGGAAGAGAAGGAGGAGGAGA TTCTCTTGCTGCACCACAAAT	$(CTC)_4$	13	2	0.583 (0.431)	199-205	15	2	202-205	17	3	202-208
est-102	*F* – CAGACACCAAAGGTGGAGAAG CGGTTTTATTTTACAGCAGTTGTG	$(AGA)_6$	16	3	0.692 (0.615)	172-187	16	1	178	16	1	178
est-103	*F* – GTCAGGAGGATAGAGGAGCTGA TTTCCCCGAATATGTAACCAAG	$(TTG)_4$	10	1	-	231	17	1	231	16	4	219-231
est-120	*T* - GGGATGTTTTGGTCGATGTTAT TGTAGACGTAGCTCGCGTAGAG	$(TC)_6$	17	1	-	236	17	2	235-237	17	6	226-238
daest-017	*H* - CTACCCTGGTTACGGAGGCTAT TACATCGTTGCCGGTTCTTTAT	$(CAG)_4$	16	5	0.500 (0.803)	217-250	17	5	193-220	14	4	192-201
daest-135A	*T* - CACGTCGCTTATAGTTTGCTTG AGAAGTAACGAGGCTTGCAGAC	$(GTC)_4$	17	3	0.813 (0.675)	181-199	14	3	181-202	16	3	181-205
daest-135B	*H* – GTCAGGAGGATAGAGGAGCTGA CCTTTTCCCCAAAAATGTAACC	$(ACC)_4$	15	1	-	235	16	1	235	17	3	223-235
daest-212	*F* – CTGTCGGACTACAACATCCAAA TCGTCGACCTTGTAGAATTTCA	$(AAG)_4$	12	2	0.824 (0.499)	156-162	16	2	150-162	17	1	162
DV-28	*H* - CGTCTTCCGTAGGCCACTC ACCATCAGTCACCTTCTGTGC	$(AG)_{14}$	16	6	0.462 (0.560)	194-206	15	3	200-212	13	11	200-382
DV-31	*H* - CTATTCTTCCTTTCGCTTACC CGTCATCAAATACGAAGTGAC	$(CT)_9$	17	2	0.308 (0.443)	130-148	7	5	138-158	11	6	132-142

[a] Locus 162 exhibits single base-pair insertion/deletion region in *D. albipictus*, hence the narrow size range relative to the number of unique alleles

Literature Cited

Alarcon-Chaidez FJ, Sun J, Wikel SK. 2007. Transcriptome analysis of the salivary glands of *Dermacentor andersoni* Stiles (Acari:Ixodidae). *Insect Biochem Mol Biol* **37**: 48-71.

Anderson JM, Sonenshine DE, Valenzuela JG. 2008. Exploring the mialome of ticks: An annotated catalogue of midgut transcripts from the hard tick, *Dermacentor variabilis* (Acari: Ixodidae). *BMC Genomics* **9**: 552.

Aubry P, Geale DW. 2011. A review of bovine Anaplasmosis. *Transbound Emerg Dis* **58**: 1-30.

Beldade P, Rudd S, Gruber JD, Long AD. 2006. A wing expressed sequence tag resource for *Bicyclus anynana* butterflies, an evo-devo model. *BMC Genomics* **7**: 130.

Dharmarajan G, Fike JA, Beasley JC, Rhodes OE. 2009. Development and characterization of 12 polymorphic microsatellite loci in the American dog tick (*Dermacentor variabilis*). *Mol Ecol Res* **9**: 131 -133.

Hamilton MB, Pincus EL, DiFiore A, Fleischer RC. 1999. Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *BioTechniques* **27**: 500-507.

Jaworski DC, Zou Z, Bowen CJ, Wasala NB, Madden R, Wang Y, Kocan KM, Jiang H, Dillwith JW. 2010. Pyrosequencing and characterization of immune response genes from the American dog tick, *Dermacentor variabilis*. *Insect Mol Biol* **19**: 617 – 630.

Jiang K, Shi Y, Zhang J, Xu N. 2011. Microsatellite primers for vulnerable seagrass *Halophila beccariia* (Hydrocharitaceae). *Am J Botany* **98**: 155-157.

Kelkar YD, Strubczewaki N, Hile SE, Chiaromonte F, Eckert KA, Makova KD. 2010. What is a microsatellite: A computational and experimental definition based upon repeat mutational behaviour at A/T and GT/AC repeats. *Genome Biol Evol* **2**: 620-635.

Leo SST, Pybus MJ, Sperling FAH. 2010. Deep mitochondrial DNA lineage divergences within Alberta populations of *Dermacentor albipictus* (Acari: Ixodidae) do not indicate distinct species. *J Med Entomol* **47**:565-574.

Lumley LM, Sperling FAH. 2011. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). *Mol Phylogenet Evol* **58**: 232-243.

Park SDE. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection [Ph.D. thesis] University of Dublin.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Heredity* **86**: 248-249.

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Mol Ecol Res* **8**: 103-106.

Rozen S, Skaletsky HJ. 2000. Primer3 on the WWW for general users and for biologist programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology (*eds* Krawetz S, Misener S). Humana Press, Totowa, New Jersey, pp. 365-386.

Samuel WM. 2004. White as a Ghost: Winter Ticks and Moose. Federation of Alberta Naturalists, Edmonton, Alberta, Canada.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotech* **18**: 233-234.

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**: 535–538.

CHAPTER THREE

Extensive sampling and additional genetic markers confirm *Dermacentor albipictus* (Acari: Ixodidae) as a single species

Introduction

Implementation of effective targeted pest control requires quick, reliable identification of pest species (Rosen 1986, Ball and Armstrong 2008). Unfortunately, identifications based on morphology are not always reliable due to the presence of cryptic species (Bickford *et al.* 2007). Furthermore, species identification based on morphology is often dependent on a variety of factors including the investigator's familiarity with the organism, specimen quality, and the life stage being identified (Hebert *et al.* 2003). Examination of parasite biology and behaviour (geographical distribution, host exploited, varying pheromone-induced responses, pest phenology, and symbiont presence) may provide alternate methods to pest identification (*e.g.* spruce budworm complex: Lumley and Sperling 2011). However, these alternatives are applicable only if the traits can be definitively associated with the species. In recent years, DNA sequencing has become indispensible for efficient delimitation and identification of important medical and agricultural pests (McManus and Bowles 1996, Gasser 1999, Armstrong and Ball 2005).

Using molecular techniques to identify pest species allows us to overcome problems with specimen quality and size, and may potentially differentiate cryptic species. The latter is particularly important for identifying closely related and morphologically similar organisms that exhibit varying efficiency in causing or transmitting diseases (*e.g.* Maingon *et al.* 2008, Estrada-Peña *et al.* 2009). However, caution must be exercised when using a single molecular marker to delimit and identify pest species. Limited knowledge on intra-specific genetic variation (Rubinoff *et al.* 2006, Ekrem e*t al.* 2007, Galtier *et al.* 2009) can potentially lead to erroneous delimitation (Song *et al.* 2008, Sperling and Roe 2009) and misidentification of pest species, which will in turn hinder monitoring

and control efforts (*e.g.* Meier *et al.* 2006). Extensive sampling across a species' geographical range and genome allows us to account for as much intra-specific genetic diversity as possible (Elias *et al.* 2007). Such genetic information can be applied in combination with other identification methods to delimit and identify pest species via an integrative approach (Wiens 2007, Schlick-Steiner *et al.* 2010).

The winter tick, *Dermacentor albipictus* (Packard), is an important veterinary parasite in North America. It is associated with severe pathology in its ungulate hosts (McLaughlin and Addison 1986, Glines and Samuel 1989, Anderson 2002, Samuel 2004) and is known to transmit anaplasmosis (Aubry and Geale 2011). The winter tick was originally described as two distinct species before observations on its ecology, life history, and hybridisation experiments resulted in both species being synonymised under *D. albipictus* (Cooley 1938, Ernst and Gladney 1975). However, recent genetic studies on *D. albipictus* revealed that this obligate ectoparasite exhibits extensive mitochondrial variation that suggested the presence of a species complex (Crosbie *et al.* 1998, Leo *et al.* 2010). However, tick specimens in these studies either consisted of small sample sizes, or were collected within a limited geographical region. This limited sampling decreases the potential overall coverage of intra-specific genetic diversity in *D. albipictus* and may restrict our understanding of and our ability to delimit its species boundaries.

In this study, my goal is to re-examine the species boundaries of *D. albipictus* by increasing both genomic and geographical sampling of genetic diversity in the species. I sampled extensively across North America and used both sequence and microsatellite markers. Species boundaries of *D. albipictus* relative to two other partially sympatric *Dermacentor* species were determined based on congruence between molecular markers and other species traits (*e.g.* tick phenology, collection localities, host animals, and symbiont presence/absence) (Hillis 1987, Jackson *et al.* 2002, Rissler and Apodaca 2007). Effects of host species and geographical locality on tick genetic variation were further investigated in Chapter 4.

Material and Methods

Specimens and DNA Extraction

Dermacentor specimens were collected by collaborators from various localities in North America (Figure 3-1) and specimens were identified to species based on morphology (Yunker *et al.* 1986). Of the 649 tick specimens collected, 49 were identified as *Dermacentor variabilis* (Say), 45 as *D. andersoni* Stiles, and 555 as *D. albipictus* (Packard). All specimens were stored in 95% ethanol at room temperature or -20°C. Specimens were cut longitudinally, slightly off the midline using sterilized razor blades. The larger sections were stored as vouchers in 95% EtOH at -20 ˚C, while smaller sections were ground in buffer solution using autoclaved pestles. DNA was extracted using QIAamp DNA mini kits (Qiagen, Valencia, CA). Voucher specimens are deposited in the E. H. Strickland Entomological Museum (University of Alberta, Edmonton, AB, Canada).

Sequencing and Genotyping

I sequenced regions of several genetic markers: mitochondrial 16S ribosomal RNA (16SrRNA) and cytochrome oxidase I (COI), and nuclear internal transcribed spacer 2 (ITS-2). COI has been proposed as the standard marker for species delimitation in animals (Hebert *et al.* 2003). ITS-2 and 16srRNA have previously been used in studies on *Dermacentor* phylogeny (Zahler *et al.* 1995, Zahler & Gothe 1997, Crosbie *et al.* 1998). Primer sequences and annealing temperatures used to amplify and sequence these markers are summarised in Table 3-1. All polymerase chain reactions were performed in 20µL reactions consisting of millipore water, 1X PCR buffer (containing 15µM $MgCl_2$), 2.5mM $MgCl_2$, 0.25mM of each dNTPs, 0.1µM forward primer, 0.1µM reverse primer, 0.125U/µL Taq polymerase, and 2.5µL of DNA template. Sequence contigs were created using SeqMan Pro (Lasergene® package, DNASTAR, Madison, WI) and sequence alignment was performed using Mesquite (Maddison and Maddison

2010) and CLUSTALW v.2.0.12 (Larkin *et al.* 2007) under default parameters. Sequence alignments were then assessed by eye in TextWrangler (http://www.barebones.com/products/textwrangler) and manually aligned if necessary. As both COI and 16SrRNA are mitochondrial markers, sequences from both markers were concatenated for additional analyses.

Fourteen microsatellite loci (Leo *et al.* 2011) were amplified for all *Dermacentor* specimens. Allele amplification was performed as described in Leo *et al*. (2011). Amplified products were diluted 1.5 in 10, co-loaded, and visualized on the ABI3730 capillary sequencer (Applied Biosystems, Foster City, CA), sized using GS500-TAMRA size standard (Applied Biosystems, Foster City, CA), and genotyped using *GeneMapper*® (Applied Biosystems, Foster City, CA).

Phylogenetic Analyses of Sequence Data

Maximum parsimony (MP) trees were obtained for all datasets using heuristic search strategies in PAUP* ver. 4.0b10 [Altivec] (Swofford 2002) with 1000 replicates, random sequence addition and TBR branch swapping routine. All characters were weighted equally and unordered (Fitch 1971). Branch supports were obtained via bootstrapping analyses performed in PAUP* with 1000 replicates using heuristic search strategies, simple sequence addition, and the TBR branch swapping routine.

The best evolution models and parameters for all datasets were determined via the Akaike Information Criterion (AIC) in MrModelTest ver. 2 (Nylander 2004) (Table 3-2) and applied in both maximum likelihood and Bayesian analyses.

Maximum likelihood (ML) analyses were performed in the program GARLi ver. 0.96b8 (Genetic Algorithm for Rapid Likelihood Inference) (Zwickl 2006) using 2,000,000 cycles for each sequence dataset. The best trees determined by GARLi were retained for each dataset and branch support values were obtained via bootstrapping with 1000 replicates in GARLi.

Bayesian analyses were performed in MrBayes ver.3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). All Bayesian analyses were performed with two simultaneous runs, each with four chains (one heated and three cold), and using default temperatures. All analyses were run for 2,000,000 cycles, with sampling every 500 generations, and a final burn-in of 1% (by which point stationarity had been achieved). Analyses were considered converged if the average standard deviation of split frequencies is less than 0.05 and when potential scale reduction factor (PSRF) values approached 1.00.

Analyses of Microsatellite Data

Microsatellite data from all specimens were analysed in *structure* ver. 2.3 (Pritchard *et al.* 2000) to determine the most likely number of species present that can be inferred from the microsatellite data. *Structure* is generally used to resolve genetic structure within species, but has also been used to study and delimit species boundaries in several species complexes (*e.g.* Isenegger *et al.* 2008, Pinzón and LaJeunesse 2011, Lumley and Sperling 2011). Preliminary clustering analysis was performed on *structure* using the no admixture ancestral model and the independent allele frequency model. Ten iterations for each number of species (k) set from 1 to 10 was analyzed with MCMC running for 100,000 generations and initial burnin of 10,000 generations. The most likely k-value was determined using the method described by Evanno *et al.* (2005), after which a more thorough run was performed with an initial burnin of 50,000 generations and 500,000 subsequent MCMC generations with k defined.

Genetic Differences between and within Each Dermacentor *Species*

To investigate the amount of genetic differentiation between and within each tick species, I performed independent AMOVA analyses on each molecular dataset (COI, 16SrRNA, concatenated mtDNA sequences, ITS-2, and microsatellite) in Arlequin ver 3.5 (Excoffier and Lischer 2010). Pairwise comparisons and exact tests of differentiation were computed with 100 permutations each using the distance method.

Comparing Variation between Molecular Markers

Variance partitioning analyses based on inferred mtDNA lineages, ITS-2 lineages, and microsatellite clusters were performed on all datasets as an indirect way of investigating if lineages/clusters inferred from another dataset could significantly explain observed variation in the markers-of-interest. For example, in order to investigate whether the distinct microsatellite clusters could be supported by COI data, I sorted COI sequences into groups that corresponded with microsatellite clusters determined by the *structure* program. If the variation in COI sequences corresponded with the variation in microsatellite data, I expected to observe significantly high variance percentage values (p-value < 0.05). Variance partitioning analyses were performed twice: the first included all three species of *Dermacentor* ticks in order to determine whether all molecular markers agreed on the species boundaries of *D. albipictus*, *D. andersoni*, and *D. variabilis.* The second included only *D. albipictus* specimens and was performed to examine whether different markers revealed corresponding genetic variation within *D. albipictus*. All analyses were performed in Arlequin using standard computations and 1000 permutations.

Other Traits

I further investigated the species boundaries of *D. albipictus* using traits such as tick phenology, host species, collection localities, and endosymbiont presence. Deviation from known winter tick phenology and any associations between tick genetic variation and host animal or associated endosymbionts may be indicative of a species complex or a speciation event in progress (McGovern and Hellberg 2003, Kuhlmann *et al.* 2007). Host animals that were examined include moose (*Alces alces* (Linnaeus)), elk (*Cervus elaphus canadensis* (Erxleben)), horses (*Equus ferus caballus* Linnaeus), mule deer (*Odocoileus hemionus* (Rafinesque)), and white-tailed deer (*Odocoileus virginianus* (Zimmermann)). Tick collection localities were included in the analyses to survey the effects of geographically associated variation on observed genetic variation.

Primers and amplification protocols that were used to investigate the presence of *Wolbachia* spp. and *Francisella*-like endosymbionts (FLEs) are as described on the *Wolbachia* MLST website (Baldo *et al.* 2006) and in Scoles (2004) respectively. *Wolbachia* spp. is a known reproductive parasite that can induce reproductive isolation within host species (Ahantarig and Kittayapong 2011). FLEs are endosymbionts of ticks (Scoles 2004) and may be potentially useful for delimiting *Dermacentor* species (Leo *et al.* 2010, Dergousoff and Chilton 2011). Variance partitioning analyses were performed using the AMOVA function in Arlequin to investigate which variable (host animal, collection region - as demarcated in Figure 3-1, or endosymbiont presence) best accounts for the genetic variation observed in these ticks.

Results

mtDNA sequences

Results from MP (tree score= 826), ML (Ln likelihood = -5519.44), and Bayesian inference analyses on a 658bp region of the COI gene are summarised on a maximum likelihood tree (Figure 3-2). COI sequences successfully delimited both *D. variabilis* and *D. andersoni* as distinct and strongly monophyletic. The corrected average amount of nucleotide differences within *D. variabilis* and *D. andersoni* were 1.263% and 1.161% respectively. There was substantial genetic differentiation between *D. andersoni* and *D. variabilis* COI sequences (6.492%). On the other hand, COI sequences revealed the presence of two well supported lineages in the winter tick (Figure 3-2). The corrected average amount of nucleotide difference between both *D. albipictus* lineages was 6.294%, with an average of 0.472% within Lineage 1, and 0.514% within Lineage 2. There was also a significantly large amount of genetic differentiation between the two *D. albipictus* lineages based on F_{ST} calculations (Table 3-3). Both COI lineages were also significantly different from *D. andersoni* and *D. variabilis* (Table 3-3).

Results from phylogenetic analyses (MP tree score = 468, ML Ln likelihood = -2676.43) of a 495bp region of the 16SrRNA gene were similar to that from COI and are summarised in Figure 3-3. *D. andersoni* and *D. variabilis* were clearly delimited as monophyletic and strongly supported (Figure 3-3) with a corrected average nucleotide difference of 0.894% within *D. variabilis*, and 0.224% within *D. andersoni*. Both *D. andersoni* and *D. variabilis* were significantly different from each other based on F_{ST} –values (Table 3-3), with a corrected average sequence difference of 3.167%. *D. albipictus* once again exhibited the presence of two distinct lineages (Figure 3-3; Crosbie *et al.* 1998, Leo *et al.* 2011). There was significant genetic variation between both lineages (Table 3-3), with a corrected average sequence difference of 2.501% (0.436% within Lineage 1, 0.649% within Lineage 2). There was significant genetic differentiation between 16SrRNA lineages and both *D. andersoni* and *D. variabilis* (Table 3-3).

Concatenated COI and 16SrRNA sequences revealed *D. albipictus* to be monophyletic, albeit with weak bootstrap support values (MP tree score = 796, ML Ln likelihood = -5856.97). The two lineages were still present and strongly supported (Figure 3-4). AMOVA analyses and exact tests of differentiation of the concatenated mtDNA sequences revealed little nucleotide variation within *D. albipictus* lineages (average corrected nucleotide variation in Lineage 1= 0.806%; Lineage 2= 0.734%), *D. andersoni* (0.636%), and *D. variabilis* (1.328%). *D. andersoni* and *D. variabilis* were genetically different from each other (Table 3-3) (~5.428%). Based on F_{ST} calculations, mtDNA lineages in *D. albipictus* exhibited significant genetic differences between each other, against *D. andersoni* and against *D. variabilis* (Table 3-3).

Phylogenetic analyses (MP tree score = 182, ML Ln likelihood = -1556.15) on a 495 bp region of ITS-2 gave a topology in which all three *Dermacentor* species were strongly monophyletic (bootstrap support values >70). There were little variation among individuals within species (Figure 3-5; corrected average sequence difference within species: *D. albipictus* = 0.022%, *D. andersoni* =

0.368%, *D. variabilis* = 0.361%). All three species of *Dermacentor* ticks exhibited substantial genetic difference between species (*D. albipictus* vs. *D. andersoni* = 1.665%; *D. albipictus* vs. *D. variabilis* = 2.331%; *D. andersoni* vs. *D. variabilis* = 2.076%) and these differences were uniformly significant based on F_{ST} calculations (Table 3-3).

Microsatellite loci

All 14 loci were polymorphic for *D. albipictus* (Table 3-4), reflecting the fact that these loci were chosen because they exhibited variation within *D. albipictus* (Chapter 2, Leo *et al*. 2011). Of the 14 loci, 3 were monomorphic for *D. andersoni*, and 2 were monomorphic for *D. variabilis* (Table 3-4). An average of 15.79 alleles per locus was obtained for *D. albipictus*, 5.43 for *D. andersoni*, and 7.21 for *D. variabilis*. Size ranges of alleles for all 14 loci differed slightly between each species with substantial overlap (Table 3-4). Results from *structure* analyses clustered the three species of *Dermacentor* ticks into five genetic groups (Ln probability= -13953.3) (Figure 3-6A). Both *D. variabilis* and *D. andersoni* individuals were clustered as discrete groups (Figure 3-6A) that were significantly different genetically (Table 3-3). On the other hand, *D. albipictus* specimens were divided into three clusters (Figure 3-6A), all of which exhibited significant genetic differences among themselves based on F_{ST} calculations (Table 3-3). Genetic differences among *D. albipictus* clusters were less than those observed between tick species (Table 3-3).

Agreement between molecular markers

Variance partition analyses revealed varying degrees of agreement between molecular markers when examined at the species level and within *D. albipictus* alone (Table 3-5). When all three *Dermacentor* species were included in the analyses, mitochondrial lineages significantly explained 27.40% of the variation observed in microsatellite data and 91.53% of sequence variation in ITS-2 sequences (Table 3-5). ITS-2 species lineages significantly explained the observed variation in all mitochondrial datasets (43.41 to 70.91%, Table 3-5).

ITS-2 species lineages also accounted for 34.89% of the variation observed in microsatellite data (Table 3-5). Microsatellite clusters significantly explained 87.41% of the variation in ITS-2 sequences, as well as the variation in mitochondrial sequences (30.94 to 53.44%, Table 3-5).

The two *D. albipictus* mtDNA lineages failed to significantly account for variation observed in *D. albipictus* microsatellite data and ITS-2 sequences (Table 3-5). On the other hand, microsatellite clusters significantly explain small amounts (1.60 – 6.87%) of the variation observed in both mtDNA and ITS-2 sequences (Table 3-5). I did not analyse the effect of ITS-2 lineages on mitochondrial markers and microsatellite data as there was only one ITS-2 lineage in *D. albipictus.*

Other species traits

According to collection records, the majority of winter ticks sampled were adults (91.51%), with larvae (2.39%), nymphs (3.98%), and eggs (2.12%) making up smaller proportions. Adult winter ticks were collected primarily from January to May, whereas eggs, larvae, and nymphs were collected in May, October, and from February to March respectively. Collection dates for all winter tick specimens, regardless of life stages, fell within previously established seasonality (Samuel 2004).

Larval, nymphal, and adult winter ticks were collected from elk (9.02%), moose (42.35%), horses (4.12%), white-tailed deer (5.49%), and mule deer (39.02%). Variance analyses revealed that the species of the host animal being exploited did not significantly account for variation observed in COI (% var. = 10.81, p-value = 0.403), 16SrRNA (% var. = 0.00, p-value = 0.840) or ITS-2 (% var. = 21.61, p-value = 0.095) sequences. However, host animals did significantly account for some of the variation observed in the microsatellite data (% var. = 7.37, p-value = 0.000) (see Figure 3-6C) and was further investigated in Chapter 4.

Wolbachia sp. was not detected in any *D. albipictus* specimens. However, of the 390 winter ticks tested, 70.77% were positive for FLE. Once again, variance analyses revealed that FLE presence/absence did not significantly account for variation observed in mtDNA or nuclear sequences (COI: % var. = 0.64, p-value = 0.181; 16SrRNA: % var. = 0.78, p-value = 0.210; ITS-2: % var. = -0.43, p-value = 0.547). However, it did significantly account for a very small amount (0.61%) of variation observed in microsatellite data (p-value = 0.010).

Collection locality did not have a significant association with observed mtDNA (COI: % var. = 10.01, p-value = 0.194; 16SrRNA: % var. = 10.54, p-value = 0.352) and ITS-2 variation (% var. = 2.59, p-value = 0.077). It did, however, account for a significant amount of the variation observed in microsatellite data (% var. = 10.04, p-value = 0.007) (see Figure 3-6D). The effect of geographical locality on winter tick genetic diversity was examined in greater detail in Chapter 4.

Discussion

Species boundaries of D. albipictus

Studies on *D. albipictus* life history, geographical distribution, and biology provided ample evidence supporting *D. albipictus* as an evolutionary, ecological, and reproductively isolated species under de Queiroz's (2007) unified species concept (Hooker *et al.* 1912, Cooley 1938, Samuel 2004). Results from this molecular study using two mitochondrial, one nuclear, and 14 microsatellite markers provided additional genealogical evidence supporting *D. albipictus* species status as distinct from *D. andersoni* and *D. variabilis*. All molecular markers delimited *D. albipictus*, *D. andersoni*, and *D. variabilis* with a high degree of agreement and there was little room for debate on the species boundaries among the three tick species. *D. albipictus* is therefore a discrete

species that maintains its genomic integrity with no substantial gene flow across species boundaries to *D. andersoni* and *D. variabilis* (Sperling 2003).

On the other hand, conflicting patterns of variation between molecular markers revealed complex genetic diversity within *D. albipictus*. While ITS-2 sequences revealed little to no variation among *D. albipictus* specimens (Figure 3-5), phylogenetic analyses on mtDNA indicated the presence of two lineages within *D. albipictus*, both of which were closely related to another one-host *Dermacentor* tick - *D. nitens* Neumann (Hebert *et al.* 2003) (Figures 3-2, 3-3, 3-4). Conversely, analyses on microsatellite markers suggested that *D. albipictus* consists of three distinct clusters (Roques *et al*. 2002) (Figure 3-6A). However, if *D. albipictus* truly consists of a species complex, I should obtain correspondence among lineages/clusters from the different molecular markers (Harr *et al.* 1998, Dettman *et al.* 2006), which was not the case (*e.g.* Figure 3-6B). This lack of agreement between different molecular markers indicates that while there is extensive and structured genetic diversity in *D. albipictus*, there is currently insufficient genealogical evidence to justify formally recognising components of a species complex.

Observations on winter tick phenology and comparisons between genetic variation and other traits (collection localities, hosts exploited, and endosymbiont) also revealed little evidence for the presence of cryptic species (de Queiroz 2007, Butler 2008). No winter ticks life stages, regardless of geographical locality, were collected outside of their previously established seasonality (Samuel 2004) and there was no significant correlation between sequence variation and species traits (Table 3-5).There were some associations between microsatellite variation and species traits, but these were limited (<12%) and may potentially be explained by the inherent evolutionary dynamics of microsatellite markers (Schlötterer 2000) as discussed below.

Extensive genetic variation in D. albipictus

There are many factors that may explain the presence of extensive intra-specific genetic variation in *D. albipictus*. Leo *et al.* (2010) discussed the roles hybridization, introgression, and retained ancestral polymorphism might have had in causing the variation observed in mtDNA. Varying mutation rates in different molecular markers may also account for the variation observed in each molecular marker (Brown *et al.* 1982, Moriyama and Powell 1997). However, results from this study suggested that biological or environmental factors may account for the extensive variation I observed in *D. albipictus* microsatellite data.

Results from variance partitioning analyses suggested that extensive genetic variation observed in *D. albipictus* microsatellite markers could be associated with environmentally correlated variables such as geographical locations and host preference (Figures 3-6B and C). As one-host ticks, *D. albipictus* dispersal is strongly dependent on host movement (Samuel 2004). Similarly, the intimate relationship between ticks and their hosts can promote evolution of host specificity (McCoy *et al.* 2001). With their rapid mutation rates, high levels of polymorphisms and selective neutrality (Schlötterer 2000, Kelkar *et al.* 2010), microsatellites will be more sensitive to subtle differences between tick populations. Knowledge on any associations between variation in tick microsatellite markers and factors such as endosymbiont diversity, ecoregions, climate conditions or host animals will be potentially useful for risk assessment of tick populations and for tracking sources of newly established tick populations in previously unoccupied habitats.

Conclusion

The objective of this study was to survey a greater proportion of the intra-specific genetic variation present in the winter tick, through extensive genomic and geographical sampling, in order to re-examine the species boundaries of *Dermacentor albipictus*. Based on the results of this study, I arrived at the same conclusion as Leo *et al.* (2010): *D. albipictus* is a genealogically distinct species and does not consist of a species complex. However, my expanded genomic and

geographical sampling scheme revealed the existence of large amounts of intra-specific genetic variation in each molecular marker that are not congruent with each other and have no current explanation. This variation may potentially be associated with ecological and environmental variables that I have not explored in this study. It will be instructive to further explore these associations, with the aim of providing useful information for more efficient design, implementation and monitoring of tick control and wildlife management policies.

Table 3-1. List of forward (F) and reverse (R) primers used for amplification and sequencing of cytochrome oxidase I (COI), 16S ribosomal RNA (16SrRNA), and internal transcribed spacer 2 (ITS-2). Details of primers used to amplify microsatellite markers can be found in Chapter Two (Table 2-2).

Genetic Marker	Annealing Temp.	Primer name	Sequence (5' – 3')	Source
COI	45˚C	F: TW-J-1302 R: HCO	GTTAACAAACTAGTAGCCTTCAAAG TAAACTTCAGGGTGACCAAAAAATCA	Murrell *et al.* 2000 Folmer *et al.* 1994
16SrRNA	42˚C	F: 16S+1 R: 16S-1	CCGGTCTGAACTCAGATCAAGT CTGCTCAATGATTTTTTAAATTGCTGTGG	Scoles 2004
ITS-2	52˚C	F: DAVF R: DAVR	ACGTACTTCGAAGGCAAACA TCACATATCAAGAGAGCCTT	Dergousoff & Chilton 2007

Table 3-2. Summary of the models of evolution and parameters obtained from the Akaike Information Criterion (AIC) in MrModelTest ver. 2 (Nylander, 2004) for cytochrome oxidase I (COI), 16S ribosomal RNA (16SrRNA), concatenated mtDNA and internal transcribed spacer 2 (ITS-2) sequences. These evolutionary models and parameters were used in the model-based maximum likelihood and Bayesian analyses.

	COI	**16SrRNA**	**Concatenated mtDNA**	**ITS-2**
Model of Evolution	GTR+I+Γ	HKY+Γ	HKY+I+Γ	GTR+I
-ln Likelihood	5656.01	2760.45	6018.06	1562.29
K	10	5	6	9
AIC	11332.02	5530.89	12048.12	3142.58
Base Frequencies				
A	0.3167	0.3607	0.3343	0.2357
C	0.1714	0.1327	0.1527	0.2787
G	0.1281	0.1364	0.1139	0.3131
T	0.3838	0.3703	0.3991	0.1725
Substitution Model				
Ti/Tv ratio	-	0.9799	1.5101	-
Substitution Rates				
A-C	1.0956	-	-	1.2643
A-G	4.6428	-	-	2.3988
A-T	1.2503	-	-	3.2511
C-G	1.8478	-	-	0.9467
C-T	3.8803	-	-	4.4298
G-T	1.0000	-	-	1.0000
Among-site Rate Variation				
Proportion of invariable sites (I)	0.3231	0.0000	0.4509	0.4340
Variable sites (Γ) (ɣ-distribution)	0.7739	0.7542	0.7511	Equal rates for all sites

Table 3-3. Pairwise comparisons of genetic differences between lineages/clusters/species based on phylogenetic results inferred from COI, 16SrRNA, ITS-2 sequences and 14 microsatellite markers. Statistically significant p-values are italicised and underlined.

Markers			**F_{ST}**	**p-value**
COI				
	Lineage 1	Lineage 2	0.925	*0.000*
	Lineage 1	*D. andersoni*	0.885	*0.000*
	Lineage 1	*D. variabilis*	0.887	*0.000*
	Lineage 2	*D. andersoni*	0.912	*0.000*
	Lineage 2	*D. variabilis*	0.920	*0.000*
	D. variabilis	*D. variabilis*	0.842	*0.000*
16SrRNA				
	Lineage 1	Lineage 2	0.806	*0.000*
	Lineage 1	*D. andersoni*	0.916	*0.000*
	Lineage 1	*D. variabilis*	0.853	*0.000*
	Lineage 2	*D. andersoni*	0.839	*0.000*
	Lineage 2	*D. variabilis*	0.787	*0.000*
	D. variabilis	*D. variabilis*	0.820	*0.000*
Concatenated mtDNA				
	Lineage 1	Lineage 2	0.863	*0.000*
	Lineage 1	*D. andersoni*	0.876	*0.000*
	Lineage 1	*D. variabilis*	0.831	*0.000*
	Lineage 2	*D. andersoni*	0.875	*0.000*
	Lineage 2	*D. variabilis*	0.858	*0.000*
	D. variabilis	*D. variabilis*	0.831	*0.000*
Nuclear ITS-2				
	D. albipictus	*D. andersoni*	0.971	*0.000*
	D. albipictus	*D. variabilis*	0.973	*0.000*
	D. andersoni	*D. variabilis*	0.851	*0.000*
Microsatellite				
	Cluster 1	Cluster 2	0.070	*0.000*
	Cluster 1	Cluster 3	0.112	*0.000*
	Cluster 1	*D. andersoni*	0.320	*0.000*
	Cluster 1	*D. variabilis*	0.381	*0.000*
	Cluster 2	Cluster 3	0.068	*0.000*
	Cluster 2	*D. andersoni*	0.323	*0.000*
	Cluster 2	*D. variabilis*	0.376	*0.000*
	Cluster 3	*D. andersoni*	0.262	*0.000*
	Cluster 3	*D. variabilis*	0.312	*0.000*
	D. andersoni	*D. variabilis*	0.311	*0.000*

Table 3-4. Size ranges and number of alleles obtained for each of the 14 microsatellite marker genotyped in *D. albipictus*, *D. andersoni*, and *D. variabilis*. The number in parenthesis following each size range indicates the number of alleles obtained for the locus in each species.

Locus	***D. albipictus* size range**	***D. andersoni* size range**	***D. variabilis* size range**
3B	137-177 (17)	139-161 (9)	119-165 (10)
6F	129-255 (38)	137-259 (16)	145-261 (20)
5E	186-252 (21)	183-258 (15)	177-210 (8)
162	332-370 (16)	330-370 (7)	306-376 (12)
est-068	166-205 (6)	202-205 (2)	202-208 (3)
est-102	166-187 (5)	178 (1)	178 (1)
est-103	222-264 (5)	231 (1)	219-231 (4)
est-120	230-238 (4)	235-237 (2)	226-244 (4)
daest-017	177-261 (26)	193-220 (7)	192-201 (4)
daest-135A	157-223 (12)	181-205 (4)	181-205 (4)
daest-135B	223-271 (5)	235 (1)	223-250 (7)
daest-212	156-204 (5)	150-162 (2)	162 (1)
DV-28	166-240 (21)	200-212 (3)	200-386 (13)
DV-31	106-152 (12)	138-158 (6)	132-142 (6)

Table 3-5. Results from variance partitioning analyses carried out as an indirect comparison of results inferred from each molecular marker (i.e. whether mtDNA / ITS-2 lineages and microsatellite clusters significantly explain the variation observed in other molecular markers). All variance partitioning analyses were done twice, once including all three species of *Dermacentor* ticks, and once more with only *D. albipictus* specimens. As there was only one ITS-2 lineage in *D. albipictus*, no AMOVA analysis was performed on the effects of ITS-2 lineage on *D. albipictus* mtDNA and microsatellite data. Statistical significance of high F_{ST} values (>0.10) is indicated by bolded and underlined p-values. Statistical significance of low F_{ST} values (<0.10) are bolded only. Results are considered statistically significant if p-value < 0.05.

	mtDNA lineages			**ITS-2 lineages**			**Microsatellite clusters**		
	% var.	F-stat	p-value	% var.	F-stat	p-value	% var.	F-stat	p-value
All three species									
COI	-	-	-	70.91	0.7091	**<u>0.0000</u>**	53.44	0.5344	**<u>0.0000</u>**
16SrRNA	-	-	-	43.41	0.4341	**<u>0.0000</u>**	30.94	0.3094	**<u>0.0000</u>**
Concatenated mtDNA	-	-	-	63.20	0.6320	**<u>0.0000</u>**	46.77	0.4677	**<u>0.0000</u>**
ITS-2	91.53	0.9153	**<u>0.0000</u>**	-	-	-	87.41	0.8741	**<u>0.0000</u>**
Microsatellite	27.40	0.2740	**<u>0.0000</u>**	34.89	0.3489	**<u>0.0000</u>**	-	-	-
***D. albipictus* only**									
COI	-	-	-				6.87	0.0687	**0.0000**
16SrRNA	-	-	-				4.96	0.0496	**0.0010**
Concatenated mtDNA	-	-	-				4.79	0.0479	**0.0352**
ITS-2	1.38	0.0137	0.1378				1.60	0.016	**0.0235**
Microsatellite	0.84	0.0084	0.0753				-	-	-

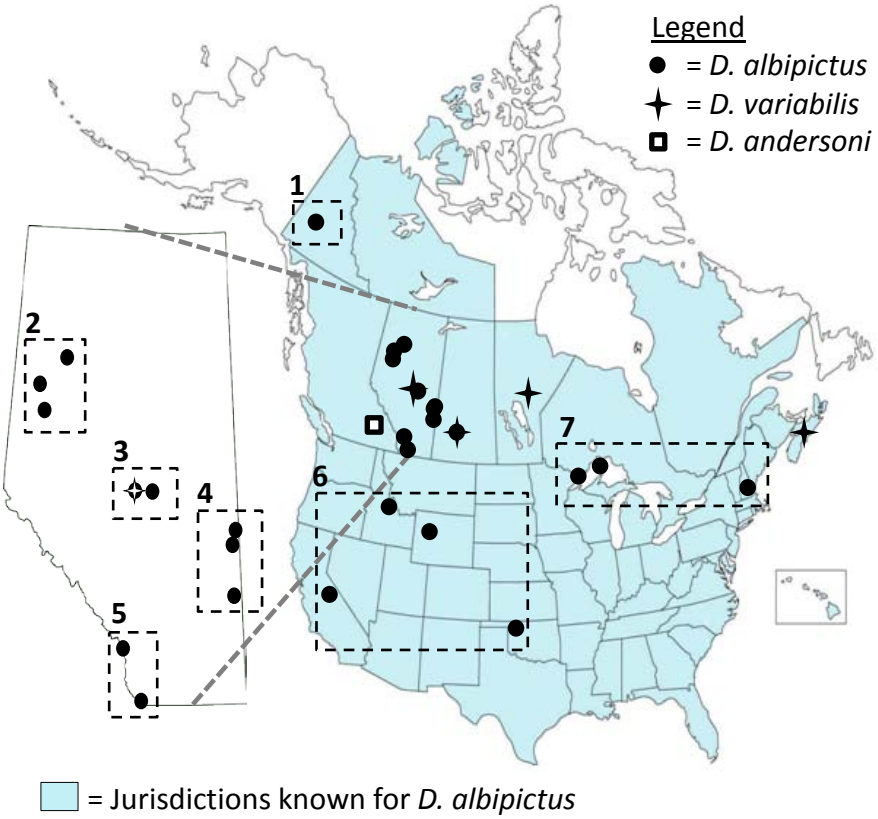
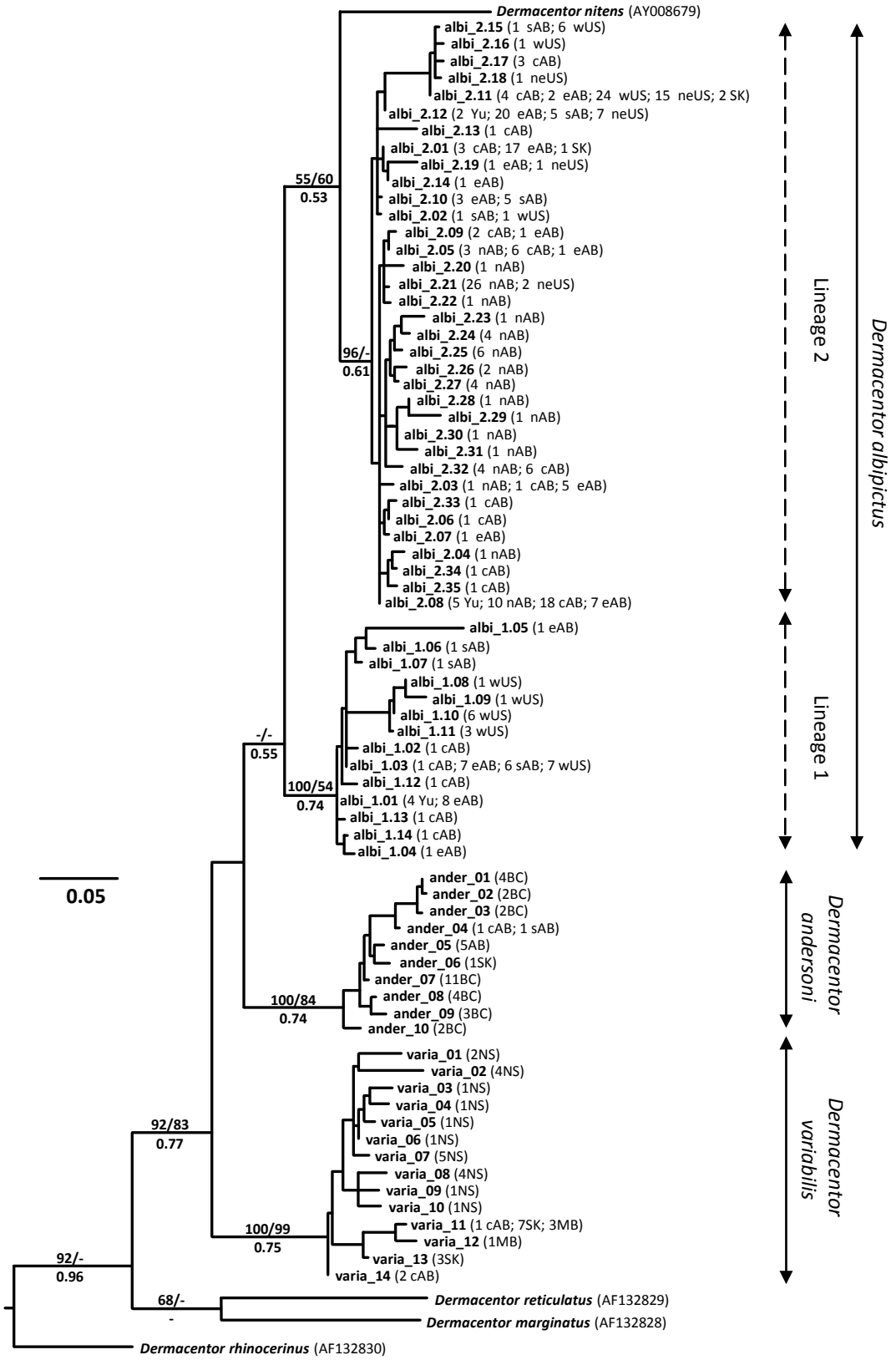


Figure 3-1. Map of collection localities for *D. albipictus* (closed circles), *D. andersoni* (open square), and *D. variabilis* (crosses) specimens across North America. The approximate jurisdictional distribution of *D. albipictus* in Canada and the United States of America was established based on literature records and is indicated by the shaded region. Collection localities were classified into geographical regions as follows: [1] Yukon (Yu) = Yukon Territory; [2] northern Alberta (nAB) = Grande Prairie, Peace River, Whitburn; [3] central Alberta (cAB) = Edmonton, Elk Island National Park; [4] eastern Alberta (eAB) = Oyen, Chauvin, Dillberry Lake Provincial Park; [5] southern Alberta (sAB) = Waterton region, Crowsnest Pass; [6] western US (wUS) = California, Idaho, Wyoming, Oklahoma; [7] northeastern US (neUS) = Minnesota, Michigan, New Hampshire.

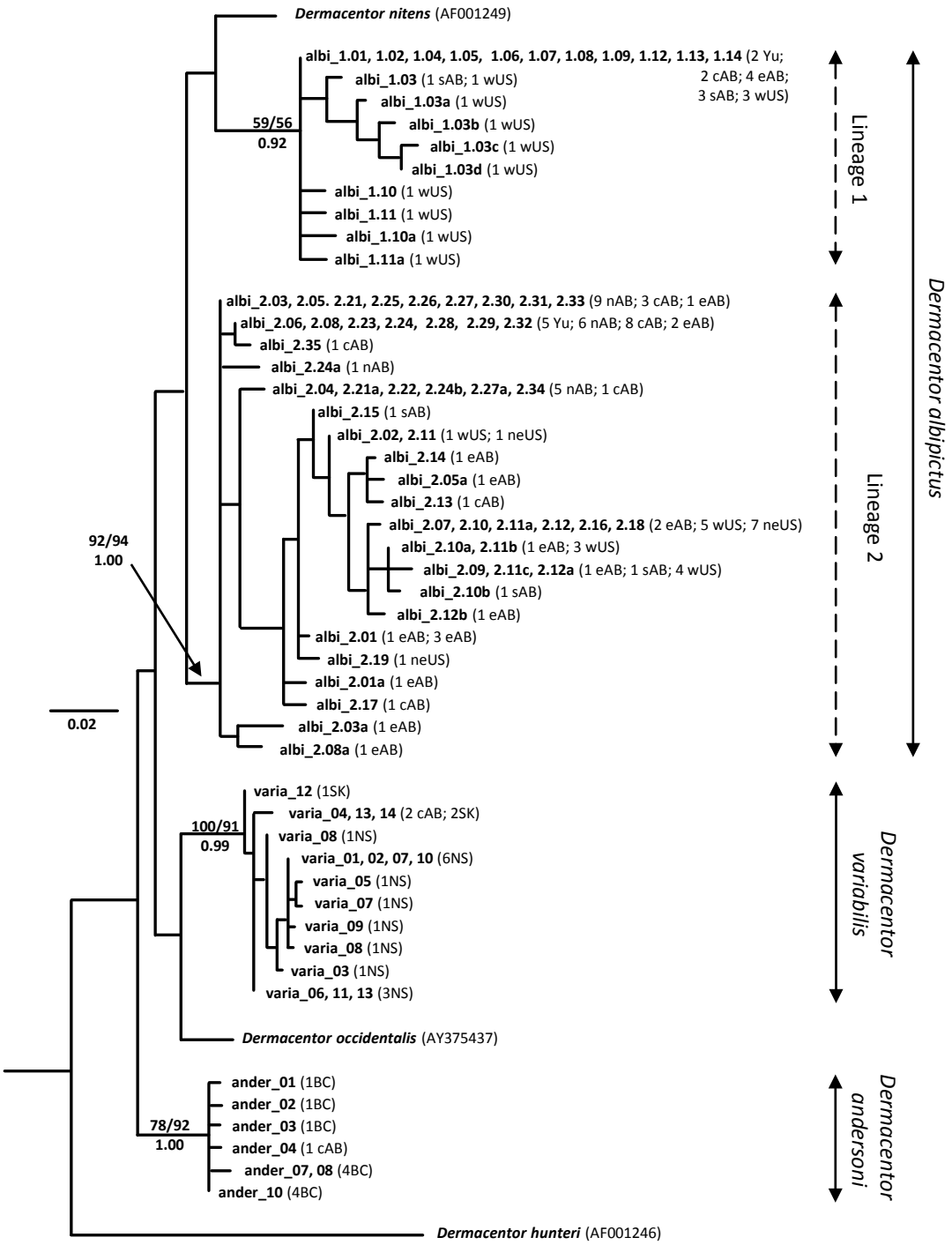
Following page:

Figure 3-2. Maximum likelihood tree for all unique mitochondrial cytochrome oxidase I (COI) haplotypes found in *Dermacentor albipictus*, *D. andersoni*, and *D. variabilis* specimens. Maximum parsimony and maximum likelihood bootstrap values (MP/ML) are indicated above the branches. Bayesian inference support values are indicated below the branches. The information in parentheses indicates the number of specimens sequenced from each region. Regional codes are the same as those in Figure 3-1, with the addition of Saskatchewan (SK), Manitoba (MB), Nova Scotia (NS), and British Columbia (BC). The names of each unique haplotype in this figure correspond with those in Leo *et al.* 2010, with new haplotypes being labelled in increasing numerical order.


Dermacentor nitens (AY008679)
albi_2.15 (1 sAB; 6 wUS)
albi_2.16 (1 wUS)
albi_2.17 (3 cAB)
albi_2.18 (1 neUS)
albi_2.11 (4 cAB; 2 eAB; 24 wUS; 15 neUS; 2 SK)
albi_2.12 (2 Yu; 20 eAB; 5 sAB; 7 neUS)
albi_2.13 (1 cAB)
albi_2.01 (3 cAB; 17 eAB; 1 SK)
albi_2.19 (1 eAB; 1 neUS)
albi_2.14 (1 eAB)
albi_2.10 (3 eAB; 5 sAB)
albi_2.02 (1 sAB; 1 wUS)
albi_2.09 (2 cAB; 1 eAB)
albi_2.05 (3 nAB; 6 cAB; 1 eAB)
albi_2.20 (1 nAB)
albi_2.21 (26 nAB; 2 neUS)
albi_2.22 (1 nAB)
albi_2.23 (1 nAB)
albi_2.24 (4 nAB)
albi_2.25 (6 nAB)
albi_2.26 (2 nAB)
albi_2.27 (4 nAB)
albi_2.28 (1 nAB)
albi_2.29 (1 nAB)
albi_2.30 (1 nAB)
albi_2.31 (1 nAB)
albi_2.32 (4 nAB; 6 cAB)
albi_2.03 (1 nAB; 1 cAB; 5 eAB)
albi_2.33 (1 cAB)
albi_2.06 (1 cAB)
albi_2.07 (1 eAB)
albi_2.04 (1 nAB)
albi_2.34 (1 cAB)
albi_2.35 (1 cAB)
albi_2.08 (5 Yu; 10 nAB; 18 cAB; 7 eAB)
55/60
0.53
96/-
0.61
Lineage 2
Dermacentor albipictus
albi_1.05 (1 eAB)
albi_1.06 (1 sAB)
albi_1.07 (1 sAB)
albi_1.08 (1 wUS)
albi_1.09 (1 wUS)
albi_1.10 (6 wUS)
albi_1.11 (3 wUS)
albi_1.02 (1 cAB)
albi_1.03 (1 cAB; 7 eAB; 6 sAB; 7 wUS)
albi_1.12 (1 cAB)
albi_1.01 (4 Yu; 8 eAB)
albi_1.13 (1 cAB)
albi_1.14 (1 cAB)
albi_1.04 (1 eAB)
-/-
0.55
100/54
0.74
Lineage 1
0.05
ander_01 (4BC)
ander_02 (2BC)
ander_03 (2BC)
ander_04 (1 cAB; 1 sAB)
ander_05 (5AB)
ander_06 (1SK)
ander_07 (11BC)
ander_08 (4BC)
ander_09 (3BC)
ander_10 (2BC)
100/84
0.74
Dermacentor andersoni
varia_01 (2NS)
varia_02 (4NS)
varia_03 (1NS)
varia_04 (1NS)
varia_05 (1NS)
varia_06 (1NS)
varia_07 (5NS)
varia_08 (4NS)
varia_09 (1NS)
varia_10 (1NS)
varia_11 (1 cAB; 7SK; 3MB)
varia_12 (1MB)
varia_13 (3SK)
varia_14 (2 cAB)
92/83
0.77
100/99
0.75
Dermacentor variabilis
92/-
0.96
68/-
-
Dermacentor reticulatus (AF132829)
Dermacentor marginatus (AF132828)
Dermacentor rhinocerinus (AF132830)

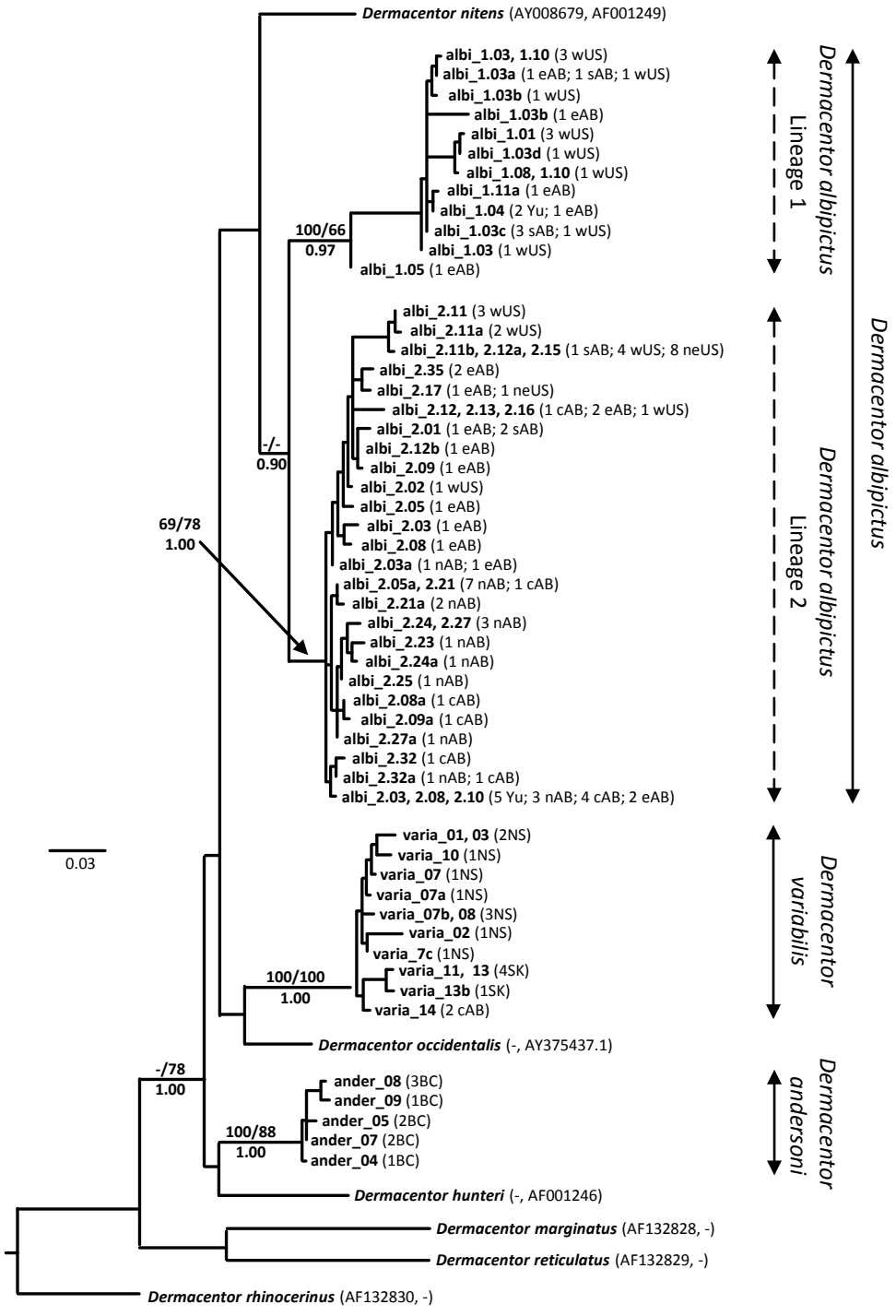
Following page:

Figure 3-3. Maximum likelihood tree for all unique mitochondrial 16S ribosomal RNA (16SrRNA) haplotypes found in *Dermacentor albipictus*, *D. andersoni*, and *D. variabilis* specimens. Clade support, haplotype information in parentheses, and locality codes are the same as those in Figure 3-2. Haplotype names in this figure correspond with those in Figure 3-2 and Leo *et al.* 2010 (multiple 16SrRNA versions of a COI haplotype are differentiated by letters).


Dermacentor nitens (AF001249)
albi_1.01, 1.02, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, 1.12, 1.13, 1.14 (2 Yu; 2 cAB; 4 eAB; 3 sAB; 3 wUS)
albi_1.03 (1 sAB; 1 wUS)
albi_1.03a (1 wUS)
albi_1.03b (1 wUS)
albi_1.03c (1 wUS)
albi_1.03d (1 wUS)
albi_1.10 (1 wUS)
albi_1.11 (1 wUS)
albi_1.10a (1 wUS)
albi_1.11a (1 wUS)
59/56
0.92
Lineage 1
albi_2.03, 2.05. 2.21, 2.25, 2.26, 2.27, 2.30, 2.31, 2.33 (9 nAB; 3 cAB; 1 eAB)
albi_2.06, 2.08, 2.23, 2.24, 2.28, 2.29, 2.32 (5 Yu; 6 nAB; 8 cAB; 2 eAB)
albi_2.35 (1 cAB)
albi_2.24a (1 nAB)
albi_2.04, 2.21a, 2.22, 2.24b, 2.27a, 2.34 (5 nAB; 1 cAB)
albi_2.15 (1 sAB)
albi_2.02, 2.11 (1 wUS; 1 neUS)
albi_2.14 (1 eAB)
albi_2.05a (1 eAB)
albi_2.13 (1 cAB)
albi_2.07, 2.10, 2.11a, 2.12, 2.16, 2.18 (2 eAB; 5 wUS; 7 neUS)
albi_2.10a, 2.11b (1 eAB; 3 wUS)
albi_2.09, 2.11c, 2.12a (1 eAB; 1 sAB; 4 wUS)
albi_2.10b (1 sAB)
albi_2.12b (1 eAB)
albi_2.01 (1 eAB; 3 eAB)
albi_2.19 (1 neUS)
albi_2.01a (1 eAB)
albi_2.17 (1 cAB)
albi_2.03a (1 eAB)
albi_2.08a (1 eAB)
Lineage 2
Dermacentor albipictus
92/94
1.00
0.02
varia_12 (1SK)
varia_04, 13, 14 (2 cAB; 2SK)
varia_08 (1NS)
varia_01, 02, 07, 10 (6NS)
varia_05 (1NS)
varia_07 (1NS)
varia_09 (1NS)
varia_08 (1NS)
varia_03 (1NS)
varia_06, 11, 13 (3NS)
100/91
0.99
Dermacentor variabilis
Dermacentor occidentalis (AY375437)
ander_01 (1BC)
ander_02 (1BC)
ander_03 (1BC)
ander_04 (1 cAB)
ander_07, 08 (4BC)
ander_10 (4BC)
78/92
1.00
Dermacentor andersoni
Dermacentor hunteri (AF001246)

Following page:

Figure 3-4. Maximum likelihood tree for the concatenated mitochondrial markers (COI and 16SrRNA) obtained from *Dermacentor albipictus*, *D. andersoni*, and *D. variabilis* specimens. Clade support, haplotype information in parentheses, and locality codes are the same as those in Figure 3-2. Haplotype names in this figure correspond with those in Figure 3-2 and Leo *et al.* 2010.


Dermacentor nitens (AY008679, AF001249)
albi_1.03, 1.10 (3 wUS)
albi_1.03a (1 eAB; 1 sAB; 1 wUS)
albi_1.03b (1 wUS)
albi_1.03b (1 eAB)
albi_1.01 (3 wUS)
albi_1.03d (1 wUS)
albi_1.08, 1.10 (1 wUS)
albi_1.11a (1 eAB)
albi_1.04 (2 Yu; 1 eAB)
albi_1.03c (3 sAB; 1 wUS)
albi_1.03 (1 wUS)
albi_1.05 (1 eAB)
100/66
0.97
-/-
0.90
69/78
1.00
albi_2.11 (3 wUS)
albi_2.11a (2 wUS)
albi_2.11b, 2.12a, 2.15 (1 sAB; 4 wUS; 8 neUS)
albi_2.35 (2 eAB)
albi_2.17 (1 eAB; 1 neUS)
albi_2.12, 2.13, 2.16 (1 cAB; 2 eAB; 1 wUS)
albi_2.01 (1 eAB; 2 sAB)
albi_2.12b (1 eAB)
albi_2.09 (1 eAB)
albi_2.02 (1 wUS)
albi_2.05 (1 eAB)
albi_2.03 (1 eAB)
albi_2.08 (1 eAB)
albi_2.03a (1 nAB; 1 eAB)
albi_2.05a, 2.21 (7 nAB; 1 cAB)
albi_2.21a (2 nAB)
albi_2.24, 2.27 (3 nAB)
albi_2.23 (1 nAB)
albi_2.24a (1 nAB)
albi_2.25 (1 nAB)
albi_2.08a (1 cAB)
albi_2.09a (1 cAB)
albi_2.27a (1 nAB)
albi_2.32 (1 cAB)
albi_2.32a (1 nAB; 1 cAB)
albi_2.03, 2.08, 2.10 (5 Yu; 3 nAB; 4 cAB; 2 eAB)
0.03
varia_01, 03 (2NS)
varia_10 (1NS)
varia_07 (1NS)
varia_07a (1NS)
varia_07b, 08 (3NS)
varia_02 (1NS)
varia_7c (1NS)
varia_11, 13 (4SK)
varia_13b (1SK)
varia_14 (2 cAB)
100/100
1.00
Dermacentor occidentalis (-, AY375437.1)
-/78
1.00
ander_08 (3BC)
ander_09 (1BC)
ander_05 (2BC)
ander_07 (2BC)
ander_04 (1BC)
100/88
1.00
Dermacentor hunteri (-, AF001246)
Dermacentor marginatus (AF132828, -)
Dermacentor reticulatus (AF132829, -)
Dermacentor rhinocerinus (AF132830, -)
Dermacentor albipictus
Lineage 1
Dermacentor albipictus
Lineage 2
Dermacentor albipictus
Dermacentor variabilis
Dermacentor andersoni

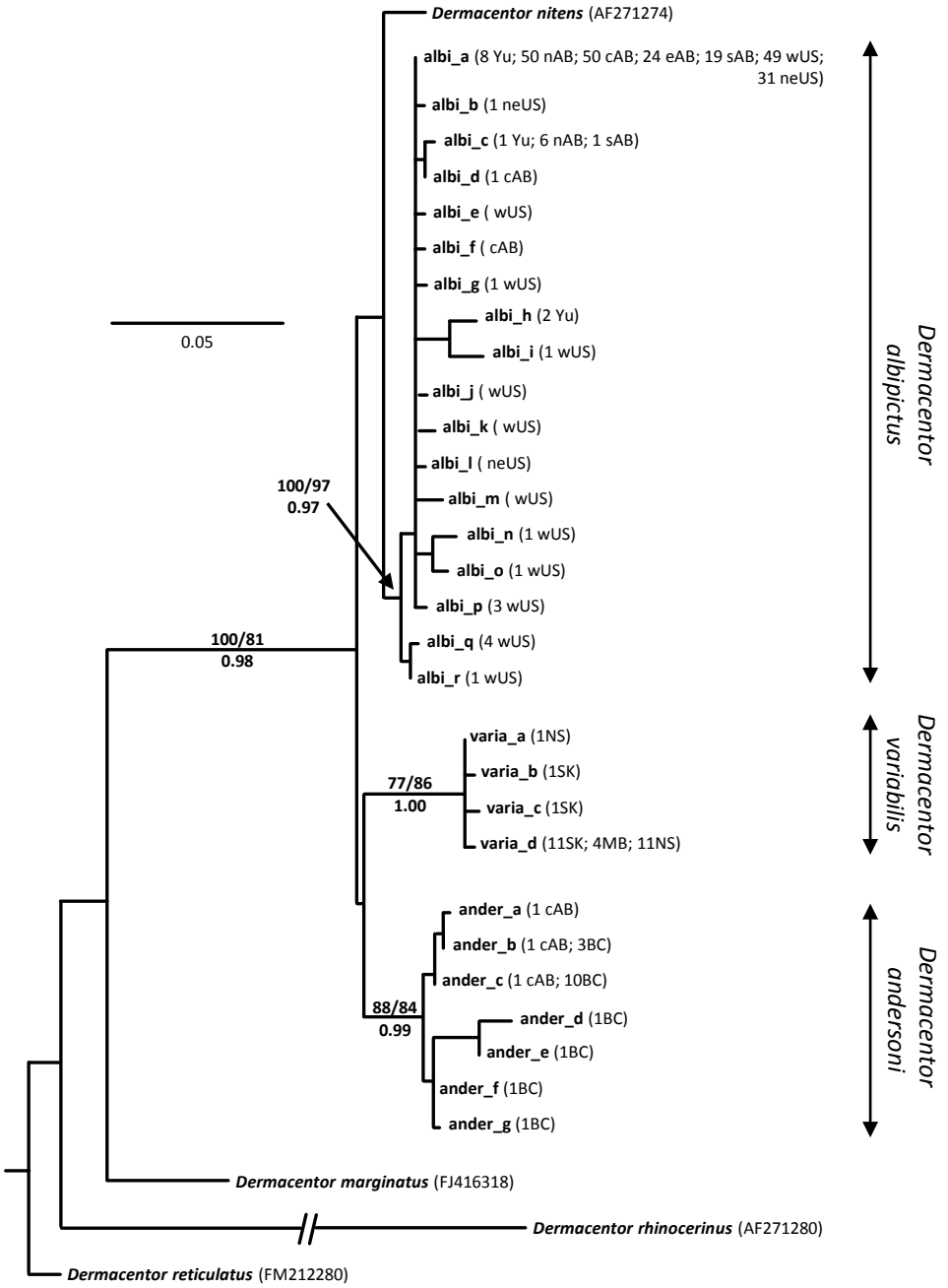


Figure 3-5. Maximum likelihood tree for all unique internal transcribed spacer 2 (ITS-2) alleles sequenced from *Dermacentor albipictus*, *D. andersoni*, and *D. variabilis* specimens. Clade support, haplotype information in parentheses and locality codes are the same as those in Figure 3-2.

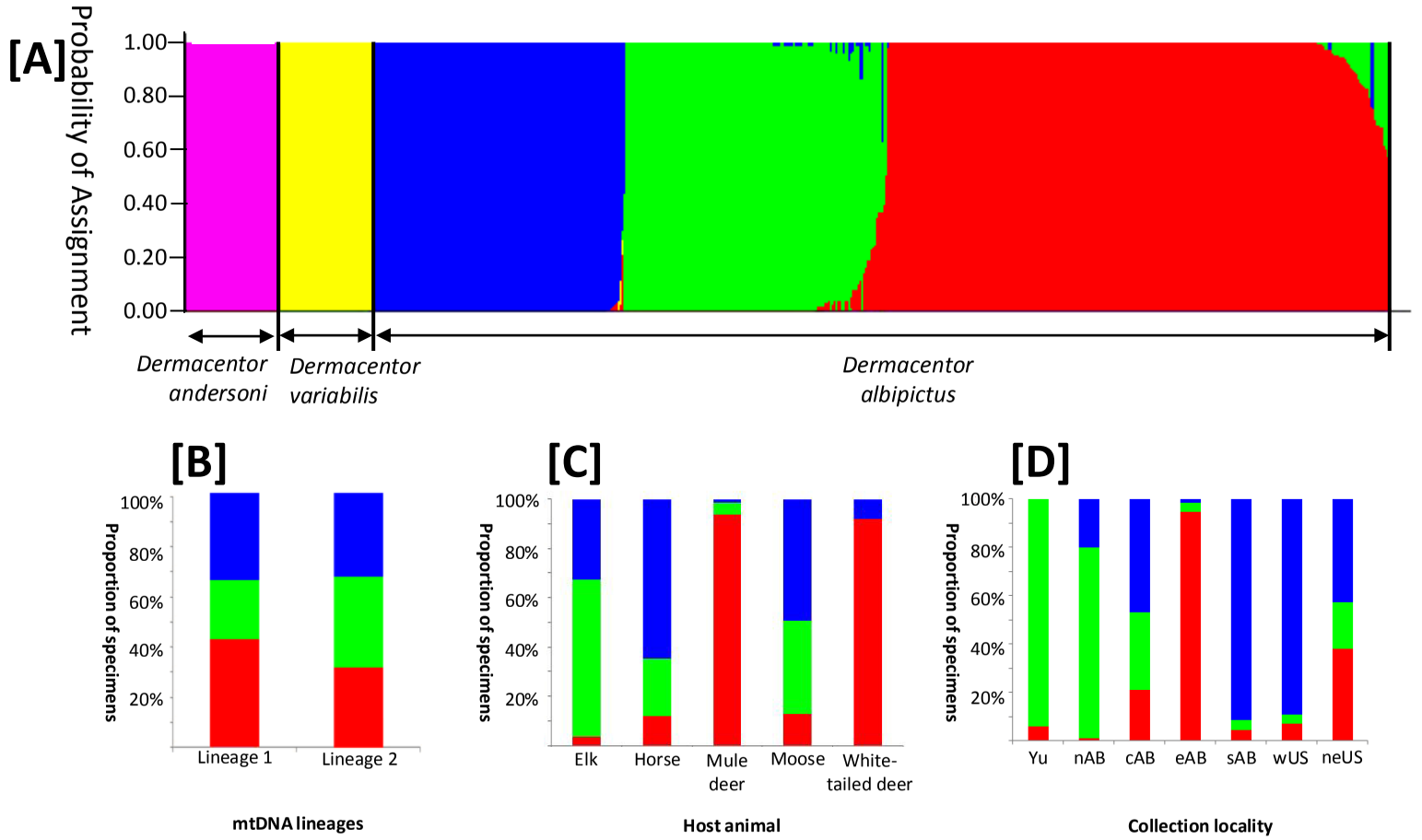


Figure 3-6. [A] Probability of simple sequence repeat assignment based on *structure* analyses (k=5, -InL = 13953.3) on 649 individuals. Analyses were performed using no admixture and independent allele frequency models. Species identification based on morphology was used as prior information. The bar graphs summarise the distribution of *D. albipictus* genetic clusters based on mtDNA lineages [B], host animal [C], and collection locality [D]. Locality codes are the same as those in Figure 3-2.

Literature Cited

Anderson JF. 2002. The natural history of ticks. *Med Clin N Am* **86**: 205-218.

Ahantarig A, Kittayapong P. 2011. Endosymbiotic *Wolbachia* bacteria as biological control tools of disease vectors and pests. *J Appl Entomol* **135**: 479 – 486.

Armstrong KF, Ball SL. 2005. DNA barcodes for biosecurity: Invasive species identification. *Phil Trans R Soc B* **360**: 1813-1823.

Aubry P, Geale DW. 2011. A review of bovine Anaplasmosis. *Transbound Emerg Dis* **58**: 1-30.

Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MC, Tettelin H, Werren JH. 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl Environ Microbiol* **72**: 7098-110.

Ball SL, Armstrong KF. 2008. Rapid, one-step DNA extraction for insect pest identification by using DNA barcodes. *J Econ Entomol* **101**: 523 – 532.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol Evol* **22**: 148-155.

Blouin MS. 2002. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. *Int J Parasitol* **32**: 527 – 531.

Brown WM, Prager EM, Wang A, Wilson AC. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. *J Mol Evol* **18**: 225-239.

Butler MG. 1982. Morphological and Phenological delimitation of *Chironomus prior sp.* n. and *c. tardus* sp. n. (Diptera: Chironomidae), sibling species from arctic Alaska. *Aquatic Insects* **4**: 219-235.

Crosbie PR, Boyce WM, Rodwell TC. 1998. DNA sequence variation in *Dermacentor hunteri* and estimated phylogenies of *Dermacentor* spp. (Acari: Ixodidae) in the New World. *J Med Entomol* **35**: 277- 288.

Cooley RA. 1938. The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation. *Natl Inst Health Bull* **171**: 59-64.

Dettman JR, Jacobson DJ, Taylor JW. 2006. Multilocus sequence data reveal extensive phylogenetic species diversity within the *Neurospora discreta* complex. *Mycologia* **98**: 436-446.

Dergousoff SJ, Chilton NB. 2007. Differentiation of three species of Ixodid tick, *Dermacentor andersoni*, *D. variabilis* and *D. albipictus*, by PCR-based approaches using markers in ribosomal DNA. *Mol Cell Probes* **21**: 343- 348.

Dergousoff SJ, Chilton NB. 2011. Associations of different genetic types of *Francisella*-like organisms with the Rocky Mountain wood tick (*Dermacentor andersoni*) and the American dog tick (*Dermacentor variabilis*) from localities near their northern distributional limits. *Appl Environ Microbiol*. doi: 10.1128/ AEM.05762-11

Ekrem T, Willassen E, Stur E. 2007. A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Mol Phylogen Evol* **43**: 530-542.

Elias M, Hill RI, Willmott KR, Dasmahapatra KK, Brower A, Mallet J, Jiggins CD. 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. *Proc R S B* **274**: 2881-2889.

Estrada-Peña A, Jongejan F. 1999. Ticks feeding on humans: A review of records on human-biting Ixodoidea with special reference to pathogen transmission. *Exp Appl Acarol* **23**: 685–715.

Estrada-Peña A, , Acevedo-Whitehouse K, Mangold AJ, Kocan KM, de la Fuente J. 2009. Phylogeographic analysis reveals association of tick-borne pathogen, *Anaplasma marginale*, MSP1a sequences with ecological traits affecting tick vector performance. *BMC Biology* **7**: 57-67.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol Ecol* **14**: 2611–2620.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res* **10**: 564-567.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specified tree topology. *Syst Zool* **2**: 406-416.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* **3**: 294–299.

Galtier N, Nabholz B, Glemin S, Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol Ecol* **18**: 4541-4550.

Gasser RB. 1999. PCR-based technology in veterinary parasitology. *Vet Parasitol* **8**: 229 – 258.

Glines MV, Samuel WM. 1989. Effect of *Dermacentor albipictus* (Acari: Ixodidae) on blood composition, weight gain and hair coat of moose, *Alces alces*. *Exp Appl Acarol* **6**: 197- 213.

Harr B, Weiss S, David JR, Brem G, Schlötterer C. 1998. A microsatellite-based multilocus phylogeny of the *Drosophila melanogaster* species complex. *Curr Biol* **8**: 1183-1186.

Hebert PDN, Cywinska A, Ball S, deWaard JR. 2003. Biological identification through DNA barcodes. *Proc R Soc Lond B* **270**: 313-321.

Hebert PDN, Gregory TR. 2005. The promise of DNA barcoding for taxonomy. *Syst Biol* **54**: 852-859.

Hillis DM. 1987. Molecular versus morphological approaches to systematic. *Ann Rev Ecol Syst* **18**: 23-42.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754-755.

Hurst GDD, Jiggins FM. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proc R Soc B* **272**: 1525-1534.

Isenegger DA, Ades PK, Ford R, Taylor PWJ. 2008. Status of the *Botrytis cinerea* species complex and microsatellite analysis of transposon types in South Asia and Australia. *Fungal Divers* **29**: 17-26.

Jackson RB, Linder CR, Lynch M, Purugganan M, Somerville S, Thayer SS. 2002. Linking molecular insight and ecological research. *Trends Ecol Evol* **17**: 409-414.

Kelkar YD, Strubczewaki N, Hile SE, Chiaromonte F, Eckert KA, Makova KD. 2010. What is a microsatellite: A computational and experimental definition based upon repeat mutational behaviour at A/T and GT/AC repeats. *Genome Biol Evol* **2**: 620-635.

Kuhlmann M, Else GR, Dawson A, Quicke DLJ. 2007. Molecular, biogeographical and phonological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae). *Org Div Evol* **7**: 155-165.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**: 2947-2948.

Leo SST, Davis C, Sperling FAH. 2011. Characterization of 14 microsatellite loci developed for *Dermacentor albipictus* and cross-species amplification in *D. andersoni* and *D. variabilis* (Acari: Ixodidae). *Conserv Genet Resour* doi: 10.1007/s12686-011-9553-x

Leo SST, Pybus MJ, Sperling FAH. 2010. Deep mitochondrial DNA lineage divergences within Alberta populations of *Dermacentor albipictus* (Acari: Ixodidae) do not indicate distinct species. *J Med Entomol* **47**: 565-574.

Lumley LM, Sperling FAH. 2011. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (*Choristoneura fumiferana*) species complex (Lepidoptera: Tortricidae). *Mol Phylogenet Evol* **58**: 232-243.

Maddison WP, Maddison DR. 2010. Mesquite: a modular system for evolutionary analysis. Version 2.73 http://mesquiteproject.org

Maingon RD, Ward RD, Hamilton JG., Bauzer LG., Peixoto, AA. 2008. The *Lutzomyia longipalpis* species complex: does population sub-structure matter to *Leishmania* transmission? *Trends Parasit* **24**: 12- 17.

McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2001. Host specificity of generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J Evol Biol* **14**: 359 – 405.

McGovern TM, Hellberg ME. 2003. Cryptic species, cryptic endosymbionts, and geographical variation in chemical defences in the bryozoan *Bugula neritina*. *Mol Ecol* **12**: 1207-1215.

McLaughlin RF, Addison EM. 1986. Tick *(Dermacentor albipictus)*-induced winter hair-loss in captive moose (*Alces alces*). *J Wildl Dis* **22**: 502- 510.

McManus DP, Bowles J. 1996. Molecular genetics approaches to parasite indentification: their values in diagnostic parasitology and systematic. *Int J Parasitol* **26**: 687-704.

Meier R, Kwong S, Vaidya G, Ng PKL. 2006. DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. *Syst Biol* **55**: 715-728.

Moriyama EN, Powell JR. 1997. Synonymous substitution rates in *Drosophila*: Mitochondrial versus nuclear genes. *J Mol Evol* **45**: 378 – 391.

Murrell A, Campbell NJH, Barker SC. 2000. Phylogenetic analyses of Rhipicephaline ticks indicate that the genus *Rhipicephalus* is paraphyletic. *Mol Phylogen Evol* **16**: 1- 7.

Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pinzón JH, LaJeunesse TC. 2011. Species delimitation of common reef corals in the genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Mol Ecol* **20**: 311 – 325.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Rissler JJ, Apocada JJ. 2007. Adding more ecology into species delimitation: Ecological niche models and phyloigeography help define cryptic species in the Black salamander (*Aneides flavipunctatus). Syst Biol* **56**: 924-942.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574. (DOI 10.1093/bioinformatics/btg180)

Roques S, Duchesne P, Bernatchez L. 2002. Potential of microsatellites for individual assignment: the North Atlantic redfish (genus *Sebastes*) species complex as a case study. *Mol Ecol* **8**: 1703-1717.

Rosen D. 1986. The role of taxonomy in effective biological control programs. *Agri Ecosyst Environ* **15**: 121-129.

Rubinoff D, Cameron S, Will K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *J Hered* **97**: 581-594.

Samuel WM. 2004. White as a Ghost: Winter Ticks and Moose. Federation of Alberta Naturalists, Edmonton, Alberta, Canada.

Savolainen V, Cowan RS, Vogler AP, Roderick GK, Lane R. 2005. Towards writing the encyclopedia of life: An introduction to DNA barcoding. *Phil Trans R Soc B* **360**: 1805-1811.

Schlötterer C. 2000. Evlutionary dynamics of microsatellite DNA. *Chromosoma* **109**: 365-371.

Scoles GA. 2004. Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. *J Med Entomol* **41**: 277-286.

Song H, Buhay J, Whiting MF, Crandall KA. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS* **105**: 13486-13491.

Swofford D. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods)**.** Sinauer, Sunderland, Massachusetts. USA.

Wiens JJ. 2007. Species delimitation: New approaches to discovering diversity. *Syst Biol* **56**: 875-878.

Yunker CE, Keirans JE, Clifford CM, Easton ER. 1986. *Dermacentor* ticks (Acari: Ixodoidea: Ixodidae) of the New World: A scanning electron microscope atlas. *Proc Entomol Soc Washington* **88**: 609-627.

Zahler M, Gothe R. 1997. Evidence for the reproductive isolation of *Dermacentor marginatus* and *Dermacentor reticulatus* (Acari: Ixodidae) ticks based on cross-breeding, morphology and molecular studies. *Exp Appl Acarol* **21**: 685-696.

Zahler M, Gothe R, Rinder H. 1995. Genetic evidence against a morphologically suggestive conspecificity of *Dermacentor reticulatus* and *D. marginatus* (Acari: Ixodidae). *J Parasit* **25**: 1413 – 1419.

Zwickl DJ. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. [Ph.D. Thesis] University of Texas at Austin.

CHAPTER FOUR

The role of host specificity versus geography in genetic differentiation between populations of the winter tick, *Dermacentor albipictus* (Acari: Ixodidae)

Introduction

Host specificity is a commonly studied aspect of host-parasite interactions. It represents the degree to which a parasite is restricted in its association with particular host species (Dick and Patterson 2007). A host specialist is a parasite whose host range is strongly limited to only one or a few closely related host species (Niogret *et al.* 2010). Conversely, a host generalist is a parasite that can feed on a broad range of suitable hosts that are available in its habitat (Niogret *et al.* 2010). The degree of specificity between parasites and their hosts can influence the establishment and sustainability of parasite populations in a region (Hoogstraal and Aeschlimann 1982, Dick and Patterson 2007). Increased understanding of degrees of host specificity in parasites will not only allow us to better understand the ecological and evolutionary aspects of host-parasite interaction, it can also serve as an important tool for efficient implementation of pest monitoring and control strategies (Stockwell and Leberg 2002).

Host specificity, or the breadth of a parasite's host range, depends on a series of ecological and behavioural factors that may influence both parasites and their hosts (Askew 1994, Shaw 1994, Sasal *et al*. 1999, Seppälä *et al*. 2008). Parasite dispersal capability, coupled with corresponding host ecology and behaviour, directly influences the number and abundance of potential host species that a parasite may encounter in a habitat (Jaenike 1990, McCoy *et al.* 2001, Dick and Patterson 2007). Parasites that have access to low numbers of potential host species are less likely to switch between hosts, leading to increased host specificity. This is especially so as parasites evolve mechanisms that allow them to more effectively parasitise the limited host choices available to them (*e.g.* overcoming specific host immune defences) (Jaenike 1990, Gandon and Van

Zandt1998, Lively and Dybdahl 2000). Given enough evolutionary time, host specialisation in combination with reproductive isolation can potentially result in parasite speciation (Bush 1994).

In this study, I investigated host specificity in an important North American tick. *Dermacentor albipictus* (Packard) (also called the winter tick) is capable of causing extensive pathology and can potentially vector a variety of disease-causing organisms (McLaughlin and Addison 1986, Glines and Samuel 1989, Anderson 2002). There has been no molecular studies investigating host preferences in *D. albipictus*, a species that commonly parasitise large ungulates such as moose (*Alces alces* (Linnaeus)), elk (*Cervus elaphus canadensis* (Erxleben)), mule deer (*Odocoileus hemionus* (Rafinesque)), white-tailed deer (*Odocoileus virginianus* Zimmermann), horses (*Equus ferus caballus* Linnaeus), cattle (*Bos primigenius* Bojanus), and mountain sheep (*Ovis ammon* (Linnaeus)) (Cooley 1938). On some occasions, this tick has also been found on humans (*Homo sapiens* Linnaeus), mountain lions (*Puma concolor* (Linnaeus)), and wolves (*Canis lupus* Linnaeus). Having such a taxonomically diverse range of potential host species is likely to impose a variety of selective pressures on the ticks, such as differential immune defences and host ecology. Additionally, as a one-host tick that spends most of its life cycle on a single host individual (Samuel 2004), reproduction and dispersal in *D. albipictus* is limited and highly host-dependent (Samuel 2004). If host populations have narrow geographical ranges that do not overlap with those of other potential host species, the winter tick's access to novel host species will be severely limited. This could result in the parasite developing specific adaptations to host species available to them in a region (Gandon and Van Zandt 1998, Lively and Dybdahl 2000).

Evolutionary change cannot occur without genetic variation. Host specialisation is usually caused or accompanied by corresponding changes in genes that allow more efficient parasitism of a particular host species (Frank 1993, Hamilton *et al*. 2005). Host-dependent mating coupled with limited parasite dispersal will further isolate parasite populations genetically. Host specificity in

parasites can therefore be studied as genetic divergences among tick populations collected from different host species (McCoy *et al*. 2001, Magalhães *et al.* 2007). Here, we used 14 microsatellite loci to examine genetic variation among ticks collected from elk, moose, mule deer, white-tailed deer, and horses across North America (Leo *et al.* 2011). If winter ticks exhibit high degrees of host specificity, we expect to observe significant genetic divergence among ticks collected from different sympatric host species. This divergence should be more pronounced than that among tick populations collected from allopatric conspecific hosts (McCoy *et al.* 2001). We also conducted isolation-by distance analyses to account for the effect of geographical distance alone on the genetic structuring of these tick populations (Magalhães *et al.* 2007).

Understanding the degree of host specificity and the presence of any geographically-associated genetic variation in *D. albipictus* will allow wildlife officials to assess the threats winter ticks may pose to potential hosts in a region and potentially determine origins of newly emerging tick populations. For example, there have been speculations that an emerging *D. albipictus* population in the Yukon Territory was either introduced into the region via elk translocation or the result of natural range expansion due to climate change (Yukon Fish and Game Association 2007, Environment Yukon 2011). Results from this study may potentially allow us to investigate the origin of this tick population in a previously naïve region as well as assess the threat it may pose to economically-important ungulates (*e.g.* moose) in the region.

Material and Methods

Specimen collection and DNA extraction

Collaborators collected *Dermacentor albipictus* specimens from various localities in North America (see Figure 3-1 in Chapter 3). Ticks were collected from a variety of host animals, including elk (*Cervus elaphus canadensis*

(Erxleben)), horses (*Equus ferus caballus* Linnaeus), moose (*Alces alces* (Linnaeus)), mule deer (*Odocoileus hemionus* (Rafinesque), white-tailed deer (*Odocoileus virginianus* Zimmermann), and mountain goats (*Oreamnos americanus* (Blainville)). All ticks were first identified to species using the key by Yunker *et al.* (1986). Detailed collection information (locality and host animal) were recorded for analyses. Genomic DNA was extracted using the QIAamp DNA mini kit (Qiagen, Valencia, CA). Remaining sections of ticks were stored as vouchers in 95% ethanol at -20˚C in the E. H. Strickland Entomological Museum at the University of Alberta (Edmonton, Alberta, Canada).

Microsatellite genotyping

Fourteen microsatellite loci developed for *D. albipictus* (Leo *et al.* 2011) were amplified in all specimens. Allele amplification was performed as described in Leo *et al.* (2011). Amplified products were diluted 1.5 in 10 for visualization on the ABI3730 capillary sequencer (Applied Biosystems, Foster City, CA) using GS500-TAMRA (Applied Biosystems, Foster City, CA) as the size standard. All specimens were genotyped using the program *GeneMapper*® (Applied Biosystems, Foster City, CA).

Analyses

Only specimens (n = 493) with good information on collection locality and host association were used in the analyses. An average of 2.49 (ranging from 1 to 14) specimens were sampled from each host individual. Winter ticks were sorted into populations based on host association and collection locality. In order ensure a reasonable sample of genetic variability, only populations containing more than five specimens were used in analyses. Tick populations collected from localities within 200km of each other were pooled, giving a final count of 14 winter tick populations as listed in Table 4-1. Linkage disequilibrium and Hardy-Weinberg equilibrium tests were performed for each population using GENEPOP ver. 4.1 (Raymond and Rousset 1995, Rousset 2008). The Excel Microsatellite

Toolkit (Park 2001) was used to obtain relevant gene diversity statistics for each population.

Microsatellite data from all specimens were analysed in *structure* ver. 2.3 (Pritchard *et al.* 2000). Preliminary clustering analysis was performed using the admixture ancestral model and correlated allele frequency model. Ten iterations for each k-value, set from 1 to 10, were analyzed with MCMC running for 100,000 generations and initial burn-in of 10,000 generations. The most likely k-value was determined using the method described by Evanno *et al.* (2005), after which a more thorough run was done with an initial burnin of 50,000 generations and 500,000 subsequent MCMC generations with k defined.

Tick populations were further sorted into groups for analyses of genetic differentiation among each host species (*e.g.* the elk group contained EL_ABC, EL_ABS, and EL_YUK (see Table 4-1)), and among collection regions where a mixture of host species was present (*e.g.* central Alberta: EL_ABC, HO_ABC, and MO_ABC)). Average number of alleles and overall Nei's gene diversities (Nei 1987) between groups were compared using Wilcoxon two-sample tests (Wilcoxon 1945) and two-sample T-tests. If host specificity was present, I expected to observe significant differences in allele counts or gene diversities among ticks collected from different hosts.

Presence and amount of genetic structuring within each group of tick populations were estimated using two different methods. The first used Wright's F-statistics (Wright 1951). Standard AMOVA, locus by locus AMOVA, and pairwise population comparisons were computed using the distance method with 10,000 permutations each, using the program Arlequin ver 3.5 (Excoffier and Lischer 2010). Population differentiation within each group was also examined by looking at genotype distribution among tick populations. Tests of genotypic differentiation were carried out in GENEPOP using unbiased log-likelihood (G) based exact tests (Goudet *et al*. 1996). Analyses in GENEPOP were carried out using Markov chain Monte Carlo methods with 100,000 iterations each.

All 493 ticks, sorted based on their collection locality, were included in isolation-by-distance analyses. Pairwise F_{ST}s for all collection localities were first obtained in Arlequin and subsequently regressed against estimated geographical distances "as the crow flies" between populations. Significance of isolation-by-distance effects were investigated using a series of Mantel tests (Mantel 1967) performed in the program R ver. 2.13.2 (http://www.R-project.org) using the library package 'ecodist'. Scatter-plots and trend-lines were also plotted in R.

Results

Genetic diversity among different populations of winter ticks

Results from *structure* analyses grouped 493 winter ticks into three genetic clusters (-Ln P= 14487.96) which appeared to correspond more with collection locality than host species (Figures 4-1, 4-2). A bar-plot, with specimens sorted according to host animals and collection regions, revealed low degrees of association between microsatellite clusters and particular host species (Figure 4-1). Conversely, when genetic clusters were mapped to collection locality, they appeared to associate strongly with particular geographical regions: 1) western US, southern and central Alberta; 2) Yukon Territory, northern and central Alberta, and a little of Michigan; 3) northeastern US, eastern Alberta, and central Alberta (Figures 4-1, 4-2). Central Alberta is the only collection region where all three genetic clusters were present (Figure 4-2).

Allele counts for winter tick populations ranged from 1 to 13 for those collected from elk, 1 to 11 for horses, 1 to 19 for moose, 1 to 14 for mule deer, and 1 to 10 for white-tailed deer. Average allele counts, gene diversity, and observed heterozygosity for each tick population are summarised in Table 4-2. Analyses using GENEPOP detected no linkage disequilibrium within or across tick populations. Two tick populations (MO_ABN and MO_WYO) showed significant deviations from Hardy-Weinberg equilibrium after Bonferroni

correction (Table 4-2). These deviations suggested the presence of processes leading to non-random associations of alleles, such as local inbreeding, fine-scaled structuring, or genetic bottlenecks (F_{IS}-values > 0, Table 4-2). Significant isolation-by-distance effects were observed when all available tick specimens were included for analysis (r = 0.686, p-value = 0.000; R^2 = 0.471, p-value = 0.000) (Figure 4-3A).

Variation within host association groups

Average allele numbers and gene diversities obtained from three populations of elk-parasitising ticks are summarised in Table 4-2. The overall average allele count in ticks collected from elk was 5.50 (±1.27) and overall gene diversity was 0.442 (±0.085). Genotypic tests (Combined test, χ^2 = infinity, df = 22, p-value = 0.0000) and F- statistics (Table 4-3) both indicated significant overall differentiation among populations of ticks collected from elk in different regions. Isolation-by-distance analysis revealed a weakly negative and statistically insignificant (r = -0.0485, p-value = 0.374; R^2 = 0.00235, p-value = 0.960) correlation between population genetic differences and geographical distance (Figure 4-3B).

Overall average allele count and gene diversity obtained from tick populations on horses in central and southern Alberta were 4.71 (±1.02) and 0.421 (±0.092) respectively (Table 4-2). Genotypic tests (Combined test, χ^2 = 40.38, df = 18, p-value = 0.0019) and F-statistics (Table 4-3) both indicated significant overall differentiation between the two tick populations. Isolation-by-distance analysis using all available ticks collected from horses in three collection localities revealed a strongly negative relationship between tick genetic differences and geographical distances (r = -0.375, p-value = 0.667; R^2 = 0.141, p-value = 1.000). However, the observed trend is statistically insignificant (Figure 4-3C).

Average allele counts obtained from seven populations of ticks collected from moose are summarised in Table 4-2. The overall average allele count was

11.29 (±2.40) and average gene diversity was 0.434 (±0.088). Genotypic tests (Combined test, χ^2 = infinity, df = 28, p-value = 0.0000) and F_{ST} calculations (Table 4-3) both indicated significant overall differentiations between ticks collected from different regions. Isolation-by-distance analyses revealed that as geographical distances increased, pairwise genetic differences increased between ticks collected from ten different localities. However, the relationship is not statistically significant (r = 0.572, p-value = 0.079, R^2 = 0.328, p-value = 0.079) (Figure 4-3D).

The average allele count and gene diversity observed from the tick population collected from mule deer and white-tailed deer in eastern AB are presented in Table 4-2. No genotypic or F-statistics tests were performed on these population as ticks from both host species were collected within a single region. Isolation-by-distance analysis revealed that for ticks collected from mule and white-tailed deer, genetic differences between populations increased as geographical distances increased (mule deer = r 0.391, p-value = 0.195; R^2 = 0.153, p-value = 0.298; white-tailed deer: r= 0.544, p-value = 0.333; R^2 = 0.296, p-value = 0.419) (Figures 4-3E and F). Both relationships were statistically insignificant.

Overall variation among host species

Ticks collected from moose tended to exhibit significantly higher overall average number of alleles per locus compared to those collected from other hosts (Table 4-4). This is possibly due to the extensive number of ticks (n = 215) sampled from moose relative to other host species. There were no significant differences in average allele numbers observed in ticks collected from elk, horse, mule deer, and white-tailed deer (Table 4-4). Wilcoxon two-sampled analyses and t-tests revealed no significant differences in tick gene diversity among host species (Table 4-4). Furthermore, pairwise genetic differences between populations collected from the same host species were not significantly different

from those observed in populations collected from different host species (Wilcoxon two-sample test: $n_1 = 25$, $n_2 = 66$, W = 1274.5, p-value = 0.266).

Variation between sympatric host species

Analyses investigating genetic divergence between ticks from different sympatric host species were performed only on populations where multiple host species were present in the same region. Collection regions that contained mixed host species were central Alberta, southern Alberta, and eastern Alberta (Table 4-1).

Genotypic differentiation tests revealed no significant genetic differences between ticks collected from mule deer and white-tailed deer in eastern Alberta (Combined test, $\chi^2 = 29.03$, df = 22, p-value = 0.144). F-statistics also revealed no significant genetic differences between ticks collected from either deer species in the region (F_{ST} value = 0.007, p-value = 0.569) (Table 4-3). However, both genotypic tests and F-statistics performed on ticks collected from elk, horses, and moose in the central Alberta region revealed significant but moderate amounts of genetic differences among populations from different host species (Combined test, χ^2 = infinity, df = 24, p-value = 0.000) (Table 3: F_{ST} value = 0.096, p-value = 0.000). Similarly, analyses on tick populations collected from horses and elk from southern Alberta revealed significant moderate genetic differences in ticks collected from different host species (Combined test, $\chi^2 = 105.76$, df = 22, p-value = 0.000) (Table 4-3: F_{ST} value = 0.139, p-value = 0.000).

Additional analyses where sufficient sampling was available (*i.e.* at least five ticks sampled per host individual) revealed no genetic structuring among ticks on each host individual in central and eastern Alberta (cAB: overall F_{ST} = -0.0875, p- value = 0.882; eAB: overall F_{ST} = -0.0799, p- value = 0.965). There was also no genetic structuring of ticks among host individuals in eastern Alberta (overall $F_{ST} < 0.000$, p- value = 0.0000). On the other hand, there was significantly moderate genetic structuring of ticks among different host individuals in central Alberta (overall $F_{ST} = 0.140$, p- value = 0.0000).

Discussion

Host preference or specificity in arthropod pests can significantly influence conservation efforts and pest control strategies. Here, we investigated the degree of host specificity in a wildlife pest of North America (the winter tick - *Dermacentor albipictus*) using fourteen polymorphic and presumably selectively neutral microsatellite loci. Comparison of gene diversity between populations of winter tick revealed significantly high amounts of variation among ticks collected from conspecific hosts in neighbouring localities (Table 4-3). Conversely, although significant amounts of genetic divergence were observed among tick populations collected from different sympatric host species, these F_{ST} values were lower than those from conspecific hosts. Results from this study suggested that geography has a larger effect on winter tick genetic variation than host specificity.

Low but significant variation in ticks from different sympatric hosts

The presence of low but significant structuring (Table 4-3: average $F_{ST} \approx 0.080$) among ticks collected from different sympatric host species in central and southern Alberta suggested that ticks might exhibit some host preference. However, the overall amount of genetic differences is lower than that observed between ticks collected from allopatric conspecific hosts (Table 4-3: average $F_{ST} \approx 0.150$). This implies that there may be other factors in the regions that may have contributed to the development of such genetic structuring.

The presence of low but significant genetic divergence observed among host species in central and southern Alberta may be explained by restricted movement of farm horses kept in fenced private properties and elk populations maintained within an enclosed national park. Fine-scaled analyses, with limited sampling, revealed that ticks collected from mule and white-tailed deer in eastern Alberta exhibited no genetic structuring among host individuals. Conversely, ticks on elk and moose individuals in central Alberta exhibited significantly moderate

genetic structuring among host individuals. The presence of physical barriers to host and parasite dispersal may explain the presence of genetic structuring between tick populations on different host individuals in central Alberta, although more extensive sampling is necessary. Barriers to dispersal may also explain the observed genetic structuring in southern Alberta.

Low amounts of specialisation in winter ticks?

There are several factors that may explain the low degree of host specificity in *D. albipictus* including host migration behaviour and distribution, human-mediated translocation of host animals (and ticks) over large distances, and winter tick host seeking cues and behaviour. This factors acting in tandem, can significantly decrease selective pressures for developing host specificity (Daszak *et al.* 2000, Dick and Patterson 2007).

Development of host specialisation is partially dependent on parasite dispersal, which is in turn dependent on host dispersal (McCoy *et al.* 2003). Ungulate hosts of winter ticks such as moose and elk are known to travel distances up to 150km annually in search of suitable grazing grounds, overwintering sites, and mates (Thomas and Irby 1990, Mauer 1998, Berger 2004, Nelson *et al.* 2004). Such extensive host dispersal can potentially weaken isolation-by distance effects between both host (Cullingham *et al.* 2010, Cullingham *et al.* 2011) and tick populations (Figure 4-3). Overlapping geographical distributions of winter tick hosts can also decrease the possibility of developing host specificity. In North America, moose, elk, mule deer, and white-tailed deer have distributions in local habitats that may overlap to varying degrees. Winter ticks in these overlapping regions are therefore likely to be exposed to a larger number of potential host species than expected, which would decrease the possibility for host specialisation.

In addition to host migratory behaviour and geographical distributions, human-mediated movement of potential winter tick hosts can overcome limitations of host and parasite dispersal (Storfer 1999, Stockwell and Leberg

2002). Privately owned horses can be transported over large geographical distances between properties. Similarly, ungulates such as elk are often translocated across North America as part of conservation efforts to replenish or reintroduce ungulates into areas where they had been extirpated (Sifton and Stephen 2002, Frair *et al*. 2010, Parks Canada 2010). If the animals were not thoroughly examined or treated for parasites before they were moved, populations of winter ticks carrying unique alleles may be unintentionally introduced into new regions, hence decreasing reproductive isolation and further suppressing development of host specificity (Daszak *et al.* 2000).

Winter ticks rely on a series of cues when host seeking, such as body heat and carbon dioxide concentration. They also climb vegetations to specific heights that, more often than not, correspond to the torso heights of large ungulates (McPherson *et al.* 2000). Winter tick questing heights therefore encompass a large variety of animals sharing similar body sizes (McPherson *et al.* 2000). Additionally, it has been noted that winter ticks that detached from their hosts prior to completion of their life cycles (either as a result of host grooming or host death) will usually endeavour to parasitise another host as soon as possible (W. Samuel pers. com. January 2012). These new hosts may include other ungulate species passing through the area or even predators and scavengers feeding on the original host. Such opportunistic parasitism behaviour in *D. albipictus* has the potential to increase parasite exposure to multiple host species, leading to decreased host specificity.

Implications for conservation and tick control

Results from this study suggested that genetic variation in winter ticks were more strongly associated with geography than host species. This knowledge on geographically-association genetic variation can be a useful tool for tracing origins of newly discovered tick populations. For example, the presence of a new population of winter tick in the Yukon Territory in the mid-1990s (Environment Yukon 2011) has been attributed to two potential sources: translocation of elk

from Elk Island National Park (Yukon Fish and Game Association 2007, Canadian Broadcasting Corporation News 2008), and natural expansion of tick populations due to global warming (Canadian Broadcasting Corporation News 2007, Environment Yukon 2011). Additional and separate analyses comparing genetic differentiations between tick populations revealed that ticks from the Yukon Territory were more genetically similar to ticks collected in central Alberta ($F_{ST} \approx 0.033$) than those collected in northern Alberta ($F_{ST} \approx 0.044$), suggesting that tick in the Canadian territory were introduced primarily from Elk Island National Park.

The low degree of host specificity in winter ticks also has important implications for conservation of ungulate wildlife in North America. If winter ticks are not limited in terms of which host species they can parasitise, introduction of winter ticks or potential hosts into an area may have severe impacts on regional ecology. For example, naïve hosts in the Yukon Territory (*e.g.* moose, caribou) that share overlapping distributions with infected elk may lack the necessary immune defences against introduced parasites that may in turn result in extensive host pathology and death (Storfer 1999, Stockwell and Leberg 2002). There is also an increased chance of cross-species transmission of tick-borne diseases that may become newly or even more firmly established in a region (Stockwell and Leberg 2002). This emphasises the need to account for all potentially susceptible host species in the region when monitoring and controlling newly established tick populations.

Conclusion

I investigated host specificity in *D. albipictus* across North America by examining genetic differences between tick populations using fourteen microsatellite loci. Based on the results of this study, I conclude that the winter ticks exhibit relatively low amounts of host specificity. Instead, results in this study indicated a greater geographical aspect to observed genetic variation in the winter tick. Low degrees of host specificity in this wildlife parasite may be

attributed to host distribution and migratory behaviour, extensive and artificial dispersal of ticks due to human interference, and the tick's generalist and opportunistic host seeking behaviour. Knowledge of the lack of host specialisation in the winter tick and association between tick genetic diversity and geographical locality constitute additional factors that should be considered in wildlife conservation policies and tick monitoring and control.

Table 4-1. List of populations into which *Dermacentor albipictus* specimens were divided for analysis of host associations, including number of specimens, host animals and collection locality. Collection localities that were less than 200km apart were pooled as a single population (i.e. northern, central, southern, and eastern Alberta). Populations with less than 5 individuals were removed from analyses.

Population	Population Code	N	Host Animals	Collection Locality/Region
1	EL_ABC	10	Elk	central Alberta, CAN
2	EL_ABS	17	Elk	southern Alberta, CAN
3	EL_YUK	16	Elk	Yukon Territory, CAN
4	HO_ABC	10	Horse	central Alberta, CAN
5	HO_ABS	8	Horse	southern Alberta, CAN
6	MO_ABC	38	Moose	central Alberta, CAN
7	MO_ABN	81	Moose	northern Alberta, CAN
8	MO_IDH	32	Moose	Idaho, USA
9	MO_MIC	11	Moose	Michigan, USA*
10	MO_MIN	20	Moose	Minnesota, USA
11	MO_NHS	13	Moose	New Hampshire, USA
12	MO_WYO	20	Moose	Wyoming, USA
13	MD_ABE	183	Mule deer	eastern Alberta, CAN
14	WT_ABE	24	White-tailed deer	eastern Alberta, CAN

northern Alberta = Grande Prairie, Whitburn, and Peace River
central Alberta = Edmonton and Elk Island National Park
southern Alberta = Crowsnest Pass and Waterton region
eastern Alberta = Dillberry Lake Provincial Park, Oyen, and Chauvin
* Ticks were collected only from Isle Royale National Park located in Michigan, USA.

Table 4-2. Summary of means of variability parameters, including standard errors (SE), and F_{IS} values for fourteen tick populations based on fourteen microsatellite loci. Population codes are given in Table 1. P-values that were significant after Bonferroni correction (p-value < 0.00357) are underlined. n_a = number of alleles, H = Nei's gene diversity, H_O = observed heterozygosity.

Group	Population Code	n_a (SE)	H (SE)	H_O (SE)	F_{IS}	p-value
Elk						
	EL_ABC	2.23 (±0.48)	0.301 (±0.089)	0.285 (±0.098)	-0.077	0.742
	EL_ABS	4.07 (±0.92)	0.487 (±0.081)	0.403 (±0.074)	0.202	0.021
	EL_YUK	3.00 (±0.52)	0.350 (±0.086)	0.289 (±0.097)	0.070	0.346
Horse						
	HO_ABC	3.29 (±0.79)	0.358 (±0.093)	0.235 (±0.080)	0.262	0.029
	HO_ABS	3.07 (±0.63)	0.389 (±0.094)	0.351 (±0.096)	0.388	0.005
Moose						
	MO_ABC	5.21 (±1.13)	0.429 (±0.082)	0.358 (±0.074)	0.117	0.036
	MO_ABN	5.43 (±1.28)	0.367 (±0.085)	0.225 (±0.069)	0.195	<u>0.000</u>
	MO_IDH	6.00 (±1.11)	0.447 (±0.088)	0.207 (±0.047)	0.025	0.418
	MO_MIC	2.50 (±0.45)	0.327 (±0.086)	0.235 (±0.070)	0.058	0.397
	MO_MIN	3.71 (±1.05)	0.368 (±0.098)	0.261 (±0.088)	-0.037	0.722
	MO_NHS	1.79 (±0.38)	0.104 (±0.059)	0.083 (±0.040)	-0.091	1.000
	MO_WYO	3.64 (±1.10)	0.358 (±0.098)	0.233 (±0.075)	0.332	<u>0.000</u>
Mule deer						
	MD_ABE	5.50 (±1.35)	0.357 (±0.091)	0.267 (±0.078)	-0.058	0.991
White-tailed deer						
	WT_ABE	4.14 (±0.89)	0.369 (±0.088)	0.271 (±0.074)	0.119	0.031

Table 4-3. F-statistics for groups of tick populations weighted/averaged over fourteen microsatellite loci. Tick populations were sorted into groups based on host association (elk, horse, moose, mule deer, and white-tailed deer) and collection regions where multiple host species were sampled (central, eastern, and southern Alberta). As mule deer and white-tailed deer were each collected from a single region (eastern Alberta), AMOVA analyses did not provide F_{ST} and F_{IT} values (shaded grey boxes). Significant p-values (< 0.05) are underlined.

Groups	F_{IS}	p-value	F_{IT}	p-value	F_{ST}	p-value
Elk	0.101 (-0.119 – 0.299)	0.008	0.241 (0.065 – 0.404)	0.000	0.156 (0.120 – 0.201)	0.000
Horse	0.259 (0.121 – 0.375)	0.000	0.341 (0.207 – 0.447)	0.000	0.111 (0.060 – 0.157)	0.010
Moose	0.311 (0.117 – 0.464)	0.000	0.436 (0.257 – 0.573)	0.000	0.182 (0.143 – 0.226)	0.000
Mule deer	0.251 (-0.013 – 0.442)	0.000				
White-tailed deer	0.271 (0.091 – 0.431)	0.000				
central Alberta	0.171 (-0.048 – 0.323)	0.000	0.250 (0.095 – 0.425)	0.000	0.096 (0.072 – 0.202)	0.000
southern Alberta	0.155 (-0.013 – 0.320)	0.000	0.272 (0.016 – 0.404)	0.000	0.139 (0.043 – 0.158)	0.000
eastern Alberta	0.250 (0.003 – 0.426)	0.000	0.255 (0.015 – 0.427)	0.000	0.007 (-0.003 – 0.016)	0.569

Table 4-4. Pairwise comparison of average allele count and gene diversity among host association groups based on Wilcoxon two-sample tests and two-sample t-tests. Significant p-values (<0.05) are underlined.

Host 1	Host 2	Average allele count				Gene diversity			
		W	p -value	t-stat	p - value	W	p -value	t-stat	p - value
Elk	Horse	196.0	0.765	0.483	0.634	201.5	0.963	0.169	0.867
Elk	Moose	152.5	*0.022*	-2.134	*0.045*	199.0	0.872	0.063	0.950
Elk	Mule deer	198.5	0.854	0.000	1.000	192.5	0.646	0.687	0.498
Elk	White-tailed deer	186.5	0.462	0.878	0.389	188.5	0.520	0.600	0.554
Horse	Moose	152.0	*0.020*	-2.520	*0.021*	192.0	0.630	-0.105	0.917
Horse	Mule deer	198.0	0.836	-0.465	0.646	202.5	1.000	0.497	0.624
Horse	White-tailed deer	196.5	0.783	0.421	0.677	195.5	0.748	0.409	0.686
Moose	Mule deer	155.5	0.031	-2.104	0.048	186.0	0.448	-0.613	0.545
Moose	White-tailed deer	143.5	0.007	2.793	0.012	184.0	0.395	-0.526	0.603
Mule deer	White-tailed deer	191.5	0.613	0.842	0.409	197.5	0.818	-0..096	0.924

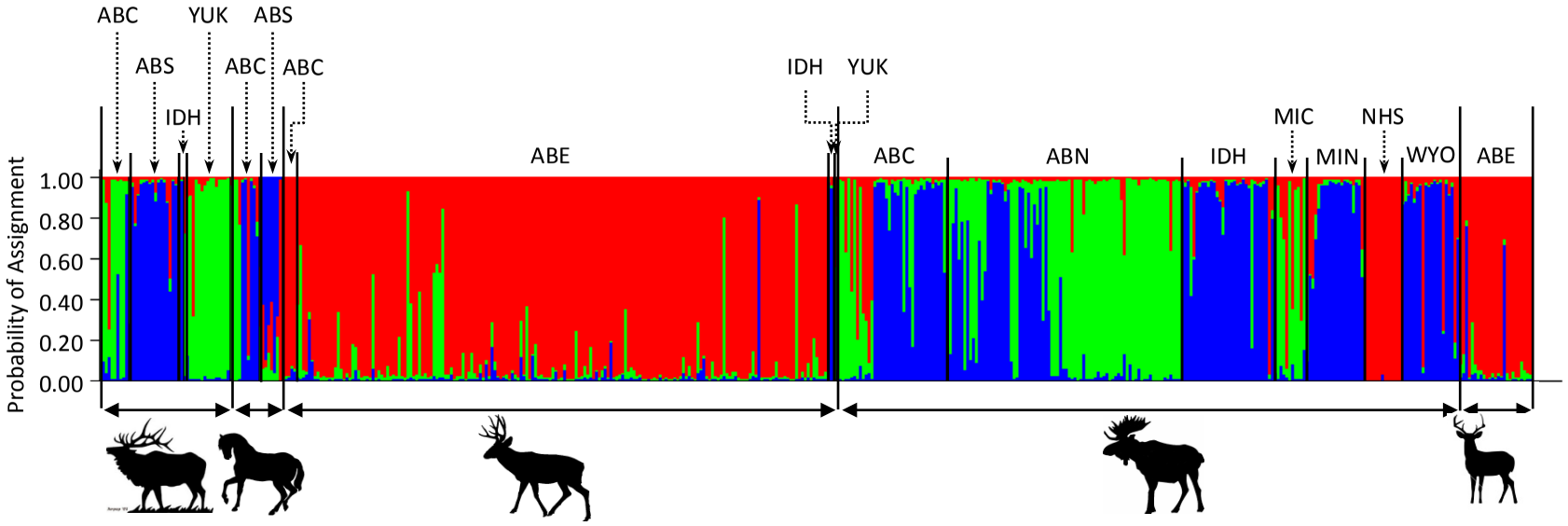


Figure 4-1. Probability of simple sequence repeat assignment based on *structure* analyses (k=3, -InL = 14487.96) on 493 *Dermacentor albipictus* ticks. Analyses were performed using admixture and correlated allele frequency models. Tick individuals in the bar-plot are arranged according to their associated ungulate host species (left to right: elk, horse, mule deer, moose, whitetailed deer) and collection regions (top labels). Codes for collection regions in this figure correspond to those in Table 4-1.

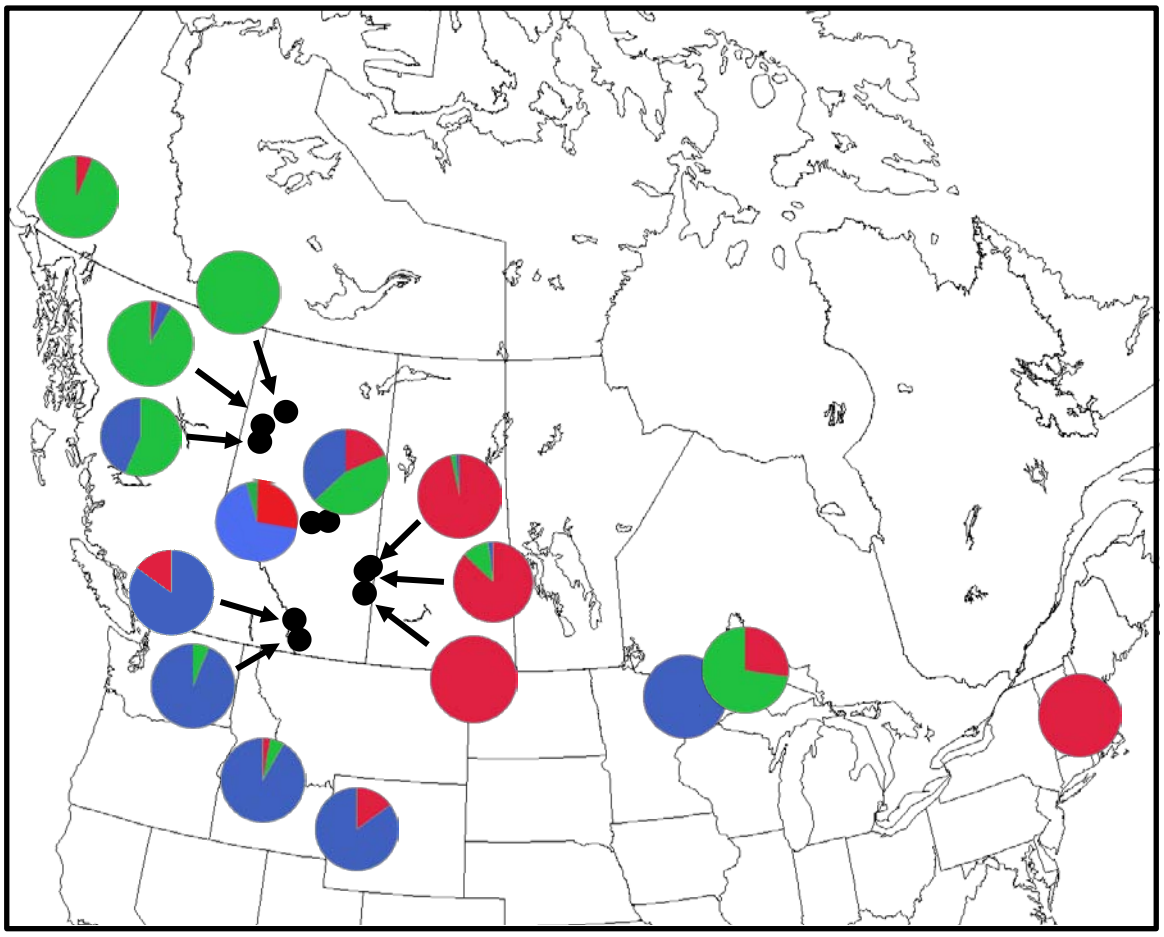


Figure 4-2. Visual representation of associations between collection locality and microsatellite clusters obtained from *structure* analyses. The pie charts indicate the proportion of specimens in each collection locality assigned to a genetic cluster and colours on the pie charts correspond to those in the bar-plot in Figure 4-1. Specimens were allocated to specific genetic clusters for which their assignment probability values were more than 50%.

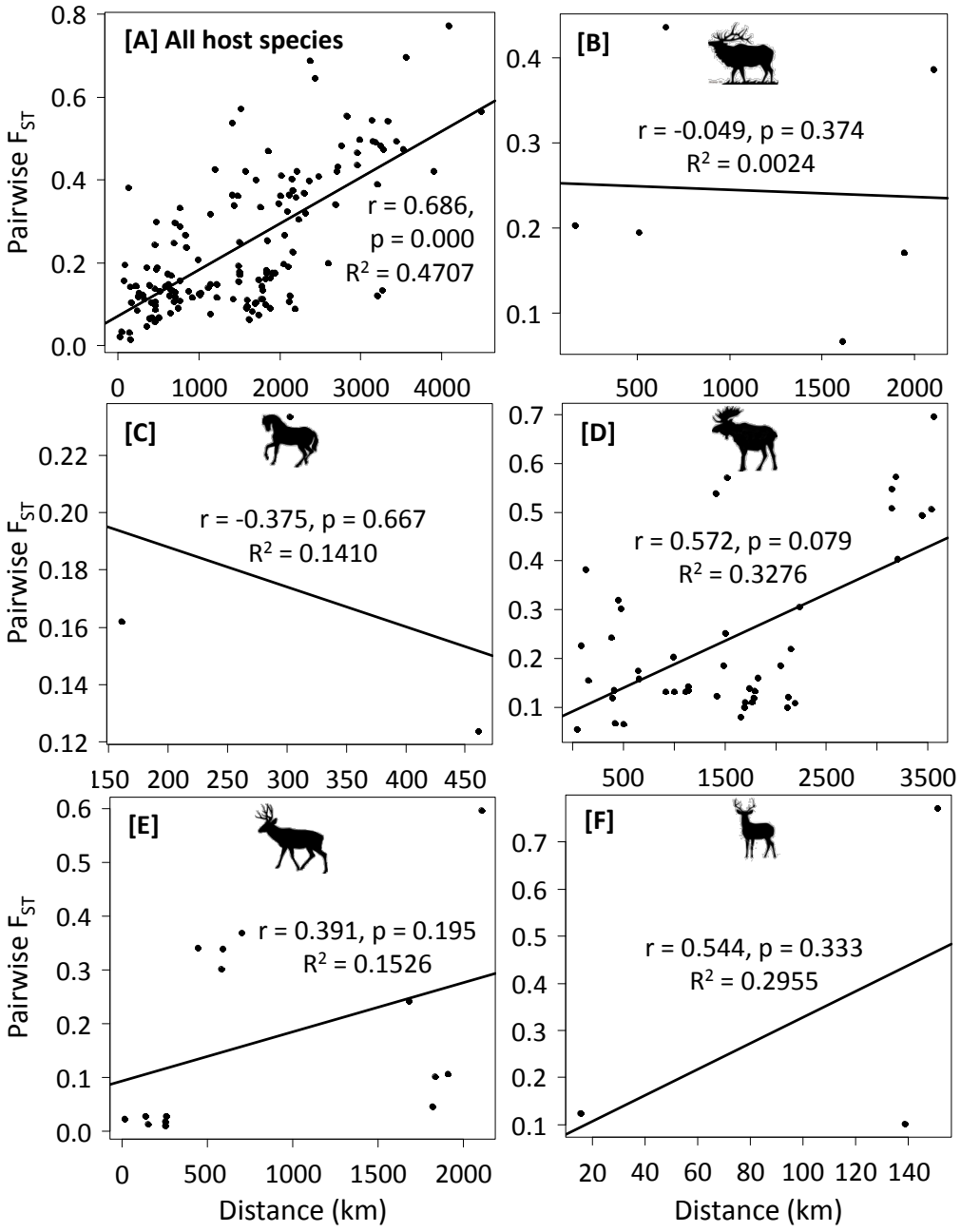


Figure 4-3. Comparison of genetic differentiation among all *Dermacentor albipictus* populations collected across North America (A), as determined by pairwise F_{ST} values regressed on geographical distance in kilometres. Ticks were also sorted according to host species for additional analyses: B) elk, C) horses, D) moose, E) mule deer, and F) white-tailed deer.

Literature Cited

Abrahamson WG, Eubanks MD, Blair CP, Whipple AV. 2001. Gall flies, inquilines, and goldenrods: A model for host-race formation and sympatric speciation. *Amer Zool* **41**: 928-938.

Anderson JF. 2002. The natural history of ticks. *Med Clin N Am* **86**: 205-218.

Askew RR. 1994. Parasitoids of leaf-mining Lepidoptera: what determines their host ranges? In: Parasitoid Community Ecology *(eds* Hawkins BA, Sheehan W). Oxford University Press. New York, pp. 177 – 202.

Berger J. 2004. The last mile: How to sustain long-distance migration in mammals. *Conserv Biol* **18**: 320 – 331.

Bernys EA, Chapman RF. 1994. Host-Plant Selection by Phytophagous Insects. Chapman & Hall, New York.

Bush GL. 1994. Sympatric speciation in animals – New wine in old bottles. *Trends Ecol Evol* **9**: 285-288.

Canadian Broadcasting Corporation News. 2007. Winter ticks invade Yukon elk herd. http://www.cbc.ca/news/canada/north/story/2007/04/18/yk-ticks.html. Retrieved 07 January 2012.

Canadian Broadcasting Corporation News. 2008. Yukon biologist unveil plan to kill winter tick infestation. http://www.cbc.ca/news/canada/north/story/2008/01/23/ticks-plan.html. Accessed 07 January 2012.

Cooley RA. 1938. The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation. *Natl Inst Health Bull* **171**: 59-64.

Cullingham CI, Merrill EH, Pybus MJ, Bollinger TK, Wilson GA, Coltman DW. 2010. Broad and fine-scale genetic analysis of white-tailed deer populations: estimating the relative risk of chronic wasting disease spread. *Evol Appl* **4**: 116 – 131.

Cullingham CI, Nakada SM, Merrill EH, Bollinger TK, Pybus MJ, Coltman DW. 2011. Multiscale population genetic analysis of mule deer (*Odocoileus hemionus hemionus*) in western Canada sheds new light on the spread of chronic wasting disease. *Can J Zool* **89**: 134 – 147.

Daszak P, Cunningham AA, Hyatt AD. 2000. Emerging infectious diseases of wildlife – Threats to biodiversity and human health. *Science* **287**: 443 – 449.

Dick CW, Patterson BD. 2007. Against all odds: Explaining high host specificity in dispersal-prone parasites. *Int J Parasitol* **37**: 871 – 876.

Environment Yukon. 2011. Winter ticks in Yukon. http://www.env.gov.yk.ca/wildlifebiodiversity/stepstomanagewintertick.php. Accessed 07 January 2102.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Mol Ecol* **14**: 2611–2620.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res* **10**: 564-567.

Frank SA. 1993. Evolution of host-parasite diversity. **47**: 1721-1732.

Friar JL, Merrill EH, Allen JR, Boyce MS. 2010. Know thy enemy: Experience affects elk translocation success in risky landscapes. *The Journal of Wildlife Management* **71**: 541 – 554.

Gandon S, Van Zandt PA (1998). Local adaptation in host-parasite interactions. *Trends Ecol Evol* **13**: 215-216.

Glines MV, Samuel WM. 1989. Effect of *Dermacentor albipictus* (Acari: Ixodidae) on blood composition, weight gain and hair coat of moose, *Alces alces*. *Exp Appl Acarol* **6**: 197- 213.

Goudet J, Raymond M, de-Meeus T, Rousset F. 1996. Testing differentiation in diploid populations. *Genetics* **144**: 1931-1938.

Hamilton R, Boots M, Paterson S. 2005. The effect of host heterogeneity and parasite intragenomic interactions on parasite population structure. *Proc Biol Sci* **272**: 1647-1653.

Hawksworth FG, Wiens D. 1996. Dwarf Mistletoes: Biology, Pathology, and Systematics. Agricultural Handbook 709. US Department of Agriculture, Washington DC, pp. 410.

Hoogstraal H, Aeschlimann A. 1982. Tick-host specificity. *Publié dans le Bulletin de la Société Entomologique Suisse* **55**: 5-32.

Jaenike J. 1990. Host specialization in phytophagous insects. *Ann. Rev. Ecol. System.* **21**: 243-73.

Krasnov BR. 2008. Functional and evolutionary ecology of fleas: A model for ecological parasitology. Cambridge University Press, New York.

Leo SST, Davis CS, Sperling FAH. 2011. Characterization of 14 microsatellite loci developed for *Dermacentor albipictus* and cross-species amplification in *D. andersoni* and *D. variabilis* (Acari: Ixodidae). *Conserv Genet Res*. doi: 10.1007/s12686-011-9553-x

Lively CM, Dybdahl MF. 2000. Parasite adaptation to locally common host genotypes. *Nature* **405**: 679-681.

Lively CM. 1989. Adaptation by a parasitic trematode to local populations of its snail host. *Evolution* **43**: 1663-1671.

Lopez Pascua L, Gandon S, Buckling A. 2012. Abiotic heterogeneity dirves parasite local adaptation in coevolving bacteria and phages. *J Evol Biol* **25**: 187-195.

Magalhães S, Forbes MR, Skoracka A, Osakabe M, Chevillon C, McCoy KD. 2007. Host race formation in the Acari. *Exp Appl Acarol* **42**: 225-238.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res* **27**: 209 – 220.

Mauer FJ. 1998. Moose migration: Northeastern Alaska to northwestern Yukon Territory, Canada. *Alces* **34**: 75 – 81.

McLaughlin RF, Addison EM. 1986. Tick *(Dermacentor albipictus)*-induced winter hair-loss in captive moose (*Alces alces*). *J Wildl Dis* **22**: 502- 510.

McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2001. Host specificity of generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J Evol Biol* **14**: 359 – 405.

McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2003. Host-dependent genetic structure of parasite populations: Differential dispersal of seabird tick host races. *Evolution* **57**: 288-296.

McPherson M, Shostak AW, Samuel WM. 2000. Climbing simulated vegetation to heights of ungulate hosts by larvae of *Dermacentor albipictus* (Acari: Ixodidae). *J Med Entomol* **37**: 114 – 120.

Nelson ME, Mech LD, Frame PF. 2004. Tracking of white-tailed deer migration by global positioning system. *J Mammalogy* **85**: 505 – 510.

Nei M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Niogret J, Lumaret J, Bertrand M. 2010. Generalist and specialist strategies in macrochelid mites (Acari: Mesostigmata) phoretically associated with dung beetles (Coleoptera: Scarabaeidae). In Trends in Acarology: Proceedings of the 12th International Congress (*eds* Sabelis MW, Bruin J). Springer Dordrecht Heidelberg, London, New York, pp. 343-347.

Park SDE. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. [Ph.D. Thesis] University of Dublin.

Parks Canada 2010. Elk Island National Park of Canada: Elk Management. http://www.pc.gc.ca/pn-np/ab/elkisland/natcul/natcul1/c/i.aspx. Accessed 11 January 2012.

Raymond M, Rousset F. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J Hered* **86**: 248–249.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Mol Ecol Resour* **8**: 103–106.

Samuel WM. 2004. White as a Ghost: Winter Ticks and Moose. Federation of Alberta Naturalists, Edmonton, Alberta, Canada.

Sasal P, Trouve S, Muller-Graf C, Morand S. 1999. Specificity and host predictability: A comparative analysis among monogenean parasites of fish. *J Anim Ecol* **68**: 437-444.

Seppälä O, Liljeroos K, Karvonen A, Jokela J. 2008. Host condition as a constraint for parasite reproduction. *Oikos* **117**: 749 – 753.

Shaw, M.R. 1994 Parasitoid host ranges. In: Parasitoid Community Ecology *(eds* Hawkins BA, Sheehan W). Oxford University Press, New York, pp. 111 – 144.

Sifton, E. and C. Stephen. 2002. Translocation of elk from Elk Island National Park, Alberta to Ontario: A risk assessment for Chronic Wasting Disease. Prepared by the Centre for Coastal Health for the Canadian Cooperative Wildlife Health Centre. pp. 27.

Stockwell CA, Leberg PL. 2002. Ecological genetics and the translocation of native fished: Emerging experimental approaches. *West N Am Natur* **62**: 32 – 38.

Storfer A. 1999. Gene flow and endangered species translocations: a topic revisited. *Biol Conserv* **87**: 173 – 180.

Thomas TR, Irby LR. 1990. Habitat use and movement patterns by migrating mule deer in southeastern Idaho. *Northwest Sci* **64**: 19 – 27.

Thompson JN. 1994**.** The Coevolutionary Process. University of Chicago Press, Chicago.

Tripet F, Christe P, Møller AP. 2002. The importance of host spatial distribution for parasite specialization and speciation: a comparative study of bird fleas (Siphonaptera: Ceratophyllidae). *J Anim Ecol* **71**: 735-748.

Wilcoxon F. 1945. Individual comparisons by ranking methods. *Biometrics Bulletin* **1**: 80–83.

Wright S. 1951. The genetical structure of populations. *Ann Eugen* **15**:323-354.

Yukon Fish and Game Association. 2007. Fact sheet: Winter tick infestation on wild elk. http://www.yukonfga.ca/display/displayDOC.asp?ID=37. Assessed 05 January 2012.

CHAPTER FIVE

General Conclusions

Thesis Summary

Parasite genetics is an emerging research field in which we can survey how heritable variation in parasites reflects vector competency and influences their interactions with other organisms and surrounding environmental conditions (Archi *et al.* 2009, Morrison *et al*. 2010, Estrada-Peña *et al.* 2009, McCoy *et al*. 2001, Louhi *et al*. 2010, Archie and Ezenwa 2011). Studies have shown that genetically similar but discrete parasites can cause different types of pathology (*e.g.* Morrison *et al.* 2009), vector different strains of disease-causing bacteria with varied efficacy (*e.g.* Maingon *et al.* 2008), and have dissimilar survival and reproductive success under different conditions (*e.g.* Agrawal 2000, Lyimo *et al*. 2012). These factors can influence how diseases and parasite populations may be maintained in a region (Fukunaga *et al.* 2000). Better understanding of pest species boundaries and how intra-specific genetic variation can influence pest behaviour is crucial for efficient parasite control. The objective of my thesis was to obtain a better understanding of genetic variation in the winter tick (*Dermacentor albipictus* (Packard)), an important veterinary parasite. In this thesis, I developed and used 14 microsatellite loci to re-examine the species boundaries of *D. albipictus* and to investigate degrees of host-specificity in this obligate ectoparasite.

In Chapter 3, I re-examined the species boundaries of *D. albipictus* via extensive genomic and geographical sampling. I determined that the winter tick consists only of a single species and is not a species complex (Chapter 3). However, the parasite exhibited different patterns of intra-specific genetic variation depending on which molecular marker one was looking at. Nuclear ITS-2 revealed little intra-specific variation in *D. albipictus* and indicated that the species is monophyletic. This may be the result of ITS-2 having the lowest mutation rate relative to all other markers used in the study (Moriyama and

Powell 1997, Kelkar *et al.* 2010) since slower evolving genes tend to produce weaker phylogenetic signals.

On the other hand, results from phylogenetic analyses on mtDNA markers (COI and 16SrRNA) revealed the presence of two strongly divergent lineages similar to those previously found in Crosbie *et al*. (1998) and Leo *et al.* (2010). Leo *et al.* (2010) suggested that the diverse genetic variation observed in winter tick mtDNA may be due to hybridization, introgression, or retained ancestral polymorphism. However, based on the lack of congruence between molecular markers and the known life history and phenology of *D. albipictus* (Hooker *et al.* 1912, Samuel 2004), recent hybridization and introgression is unlikely to explain such divergent mtDNA variation although older events of this kind remain a possibility. Retained ancestral polymorphism (whereby there was a recent merger of two previously distinct species of *Dermacentor* ticks, with ITS-2 sequences from one of the species having undergone a selective sweep and becoming established in the other species) is therefore the most plausible explanation for the presence of the two distinct mtDNA lineages. This study reiterated the challenges of delimiting and identifying species using only a single type of genetic marker (see also Elias *et al.* 2007, Galtier *et al.* 2009). An integrative multi-character approach to species delimitation and identification, as exemplified in this study, is a more reliable method for species delimitation as it not only decreases vulnerability to errors, it also allows researchers to identify new associations between genetic diversity and other variables (*e.g.* such as host animal and geography: Chapters 3 and 4).

Variance partitioning analyses in Chapter 3 suggested that extensive microsatellite variation in *D. albipictus* could be associated with factors such as geography and host species. When this was further examined in Chapter 4, I found that the winter tick exhibited low levels of specificity for any particular ungulate host species (Chapter 4). However, observed genetic differences between populations of *D. albipictus* appeared to be associated with geographical location (Chapter 4). A brief analysis in *structure* ver. 2.3 (Pritchard *et al.* 2000)

revealed three genetic clusters that were associated with three particular geographical regions: 1) western US, southern, and central Alberta; 2) Yukon Territory, northern and central Alberta, and part of Michigan; 3) northeastern US, eastern Alberta, and central Alberta (Chapter 4). The presence of all three genetic clusters in central Alberta suggests there is something evolutionarily interesting occurring in the region that deserves further research.

The presence of geographically-associated genetic variation in *D. albipictus* can be potentially useful for assessing veterinary and economic threats that particular parasite populations may present in endemic regions. It had been shown that populations of ticks inhabiting different ecoregions exhibit varying efficacy for vectoring diseases and causing pathology (Estrada-Peña *et al.* 2009, Lysyk 2010). Similarly, such geographically-associated genetic assemblages can be used to trace the origins of new tick populations in previously naïve habitats. For example, results in Chapter 4 indicated that the newly established population of winter ticks in the Yukon Territory was more genetically similar to ticks in central Alberta than northern Alberta (Chapter 4). This suggests there is a high likelihood that ticks in the northern Canadian territory were introduced from central Alberta (in particular, Elk Island National Park) when infected elk were translocated into the region. However, the difference in tick genetic similarity between regions was not statistically significant (Chapter 4); therefore I could not rule out natural range expansion due to factors like climate change as a potential cause (Ogden *et al.* 2006).

With their polymorphic nature and selective neutrality (Schlötterer 2000), microsatellites are very sensitive to subtle differences between populations and species. Microsatellites have therefore been useful for species delimitation and identification in several organisms and are particularly popular in studies on population structure and genetic diversity (*e.g.* Lumley and Sperling 2011, Feijoo *et al.* 2011, Afanas'ev *et al.* 2011). However, microsatellite markers have several limitations that can result in erroneous interpretation of results. These limitations can arise from both technical issues and biological factors.

An example of technical issues common to microsatellites is null alleles, in which an allele fails to amplify due to nucleotide substitutions at primer binding regions. This can severely influence allele frequency estimation (Pemberton *et al.* 1995). Size homoplasy, whereby alleles of the same size (electromorphs) do not share a common evolutionary history, is also another potential problem when dealing with microsatellites as they inhibit a researcher's ability to accurately compare evolutionary processes between species or populations (Estoup *et al*. 2002). Microsatellites are also limited by the fact that primers are usually species specific and will often fail to work in distantly related species (Dallimer 1999, Primmer *et al*. 2009, Zane *et al.* 2002). Attempts to genotype *Ixodes* ticks using the microsatellite loci developed in Chapter 2 gave poor results (not reported) with only 2 out of 14 loci genotyping successfully. This degree of species specificity limits a researcher's ability to perform phylogenetic studies at higher levels.

In addition to technical issues, real biological processes such as kinship or other hierarchical genetic structure can result in significant deviations from Hardy Weinberg equilibrium (Dharmarajan *et al.* 2011). Unfortunately, such heterozygote deficits are often attributed to technical issues which may in turn result in erroneous interpretations of results in population genetic studies (Dharmarajan *et al.* 2011). It is therefore important that researchers have a good understanding of the life history and biology of their study organisms and to be aware of how natural biological processes may explain unexpected patterns in their results.

Future Research

The presence of distinctly divergent mtDNA lineages and geographically-associated microsatellite variation in *D. albipictus* needs to be investigated further. This can potentially be performed via high throughput genotyping by sequencing techniques, such as RAD-sequencing (Elshire *et al*. 2011). This approach enables

researchers to sample tens of thousands of single-nucleotide polymorphisms (SNPs) simultaneously in hundreds of specimens, allowing for more extensive surveys of genetic variation. This genetic information can be used to perform comprehensive phylogenetic studies including all 34 recognised *Dermacentor* species to determine the evolutionary origin of the proposed incomplete lineage sorting in *D. albipictus* mtDNA. The same method could also be applied to investigate potential associations between winter tick genetic diversity and other ecological or environmental variables that were not examined in this thesis. Understanding these associations can potentially allow us to quantify interactions between *D. albipictus* and its surrounding environmental variables which will in turn be critical for designing and implementing efficient tick control and wildlife management strategies.

Ticks are capable of vectoring a diverse variety of diseases, such as anaplasmosis, ehrlichiosis, tularaemia, and Lyme disease (Bratton and Corey 2005). Unfortunately, due in part to difficulties in culturing microorganisms in laboratories, there have been few studies aimed at characterising the complete genetic diversity of bacterial symbionts in these parasites (*e.g.* Yuan 2010, Blomstrom 2010). Fortunately, a newly emerging bioinformatics approach known as metagenomics can enable researchers to study microorganism assemblages and genetic diversity without having to use standard culturing methods (Handelsmann 2004, Risenfeld *et al* 2004). Knowledge of associations between tick-borne bacterial genetic diversity with particular tick species, ecoregions or host animals can be critical tools for diagnosis of diseases. An extensive survey of bacteria in ticks can also potentially result in identification of previously unknown disease causing agents. Such information will be crucial for health officials when planning and establishing disease surveillance and tick control programmes.

In order to be able to establish efficient pest control strategies and to better model or predict range expansion of parasites and diseases, we need to increase our knowledge on the many interactions between host-parasite-pathogen systems and their surrounding landscape variables. This can be achieved using a newly

developed research approach known as integrated landscape genomics (James *et al.* 2011). Landscape genomics will allow us to incorporate genetic information obtained (usually via high-throughput sequencing methods) from each species in a system and relate it to other spatial variables (*e.g.* altitude, vegetation, temperature, and humidity). This allows researchers to quantify interactions among each species and their surrounding landscape that may be critical for predicting and modelling the spread of ticks and tick-borne diseases across a geographical region.

In the near future, I hope to apply an integrated landscape genetics approach (James *et al.* 2011) to investigate similarity in population structures among mammalian hosts, ticks, and disease-causing agents in Canada. Using corresponding geographical patterns of genetic variation among hosts, ticks, and pathogens will allow us to identify and explain associations between species migration and geographical heterogeneity. This will help us to better comprehend spatial population dynamics in this host-parasite-pathogen system and should be useful for modelling the spread of tick-borne diseases across the country (Bjørnstad *et al*. 1999). It may also allow us to identify biotic and abiotic variables that may influence the establishment and expansion of these diseases in Canada. Such a study exemplifies the kind of multi-disciplinary, collaborative approaches needed for advances in understanding the biology and control of complex disease systems (Ostfeld 2011).

Literature cited

Afanas'ev KI, Rubtsova GA, Shitove MV, Malinina TV, Rakitskaya TA, Prokhorovskaya VD, Shevlyakov EA, Zavarina LO, Bachevskaya LT, Chereshnev VP, Brykov VA, Kovalev MY, Shevlyakov VA, Sidorova SV, Borzov SI, Pogodin VP, Fedorova LK, Zhivotovsky LA. 2011. Population structure of chum salmon *Oncorhynchus keta* in the Russian far east, as revealed by microsatellite markers. *Rus J Mar Biol* **37**: 42 – 51.

Agrawal AA. 2000. Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology* **81**: 500 – 508.

Allan BF, Keesing F, Ostfeld RS. 2003. Effect of forest fragmentation on Lyme disease risk. *Conserv Biol* **17**: 267 – 272.

Archie EA, Ezenwa VO. 2011. Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. *Int J Parasitol* **41**: 89 – 98.

Archie EA, Luikart G, Ezenwa VO. 2009. Infecting epidemiology with genetics: a new frontier in disease ecology. *Trends Ecol Evol* **24**: 21–30.

Bjørnstad ON, Ims RA, Lambin X. 1999. Spatial population dynamics: analyzing patterns and processes of population synchrony. *Trends Ecol Evol* **14**: 427 – 432.

Blomstrom AL. 2010. Applications of viral metagenomics in the veterinary field. [PhD Thesis] Swedish University of Agricultural Sciences.

Bratton RL, Corey GR. 2005. Tick-borne disease. *Am Fam Phys* **71**: 2323-2330.

Crosbie PR, Boyce WM, Rodwell TC. 1998. DNA sequence variation in *Dermacentor hunteri* and estimated phylogenies of *Dermacentor* spp. (Acari: Ixodidae) in the New World. *J Med Entomol* **35**: 277- 288.

Dallimer M. 1999. Cross-species amplification success of avian microsatellites in the red-billed quelea *Quelea quelea*. *Mol Ecol* **8**: 695 – 697.

Dharmarajan G, Beasley JC, Rhodes Jr. OE. 2011. Heterozygote deficiencies in parsite populations: an evaluation of interrelated hypotheses in the raccoon tick, *Ixodes texanus*. *Heredity* **106**: 253 – 260.

Elias M, Hill RI, Willmott KR, Dasmahapatra KK, Brower A, Mallet J, Jiggins CD. 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. *Proc R S B* **274**: 2881-2889.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* **6**: e19379. doi:10.1371/journal.pone.0019379

Estoup A, Jarne P, Cornuet J. 2002. Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Mol Ecol* **11**: 1591 – 1604.

Estrada-Peña A, Naranjo V, Acevedo-Whitehouse K, Mangold AJ, Kocan KM, de la Fuente J. 2009. Phylogeographic analysis reveals association of tick-borne pathogen, *Anaplasma marginale,* MSP1a sequences with ecological traits affecting tick vector performance. *BMC Biology* **7**: 57-67.

Feijoo M, Lessa EP, de Castro RL, Crespo EA. 2011. Mitochondrial and microsatellite assessment of population structure of South American sea lion

(*Otaria flavescens*) in the southwestern Atlantic Ocean. *Mar Biol* **158**: 1857 – 1867.

Galtier N, Nabholz B, Glemin S, Hurst GDD. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol Ecol* **18**: 4541-4550.

Handelsman J. 2004. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev* **68**: 669-685

Hooker WA, Bishopp FC, Wood HP. 1912. The life-history and bionomics of some Northern American ticks. *U.S. Dept Agric Bur Entomol Bull* **106**: 239.

James PMA, Coltman DW, Murray BW, Hamelin RC, Sperling FAH. 2011. Spatial genetic structure of a symbiotic beetle-fungal system: Toward multi-taxa integrated landscape genetics. *PLoS ONE* **6**: doi:10.1371/journal.pone.0025359

Kelkar YD, Strubczewaki N, Hile SE, Chiaromonte F, Eckert KA, Makova KD. 2010. What is a microsatellite: A computational and experimental definition based upon repeat mutational behaviour at A/T and GT/AC repeats. *Genome Biol Evol* **2**: 620-635.

Leo SST, Pybus MJ, Sperling FAH. 2010. Deep mitochondrial DNA lineage divergences within Alberta populations of *Dermacentor albipictus* (Acari: Ixodidae) do not indicate distinct species. *J Med Entol* **47**: 565 – 574.

Louhi K, Karvonen A, Rellstab C, Jokela J. 2010. Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? *Infect Genet Evol* **10**: 1271 – 1277.

Lumley LM, Sperling FAH. 2011. Utility of microsatellites and mitochondrial DNA for species delimitation in the spruce budworm (Choristoneura fumiferana) species complex (Lepidoptera: Tortricidae). *Mol Phylogenet Evol* **58**: 232-243.

Lyimo IN, Keegan SP, Ranford-Cartwright LC, Ferguson HM. 2012. The impact of uniform and mixed species blood meals on the fitness of the mosauito vector *Anopheles gambiae s.s*: does a specialist pay for diversifying its host species diet. *J Evol Biol* **25**: 452 – 460.

Lysyk TJ. 2010. Tick paralysis caused by *Dermacentor andersoni* (Acari: Ixodidae) is a heritable trait. *J Med Entomol* **47**: 210 – 214.

Maingon RD, Ward RD, Hamilton JG, Bauzer LG, Peixoto AA. 2008. The *Lutzomyia longipalpis* species complex: does population sub-structure matter to *Leishmania* transmission? *Trends Parasit* **24**: 12- 17.

McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2001. Host specificity of generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *J Evol Biol* **14**: 359 – 405.

Morrison LJ, Tait A, McLellan S, Sweeney L, Turner CMR, MacLeod A. 2009. A major genetic locus in *Trypanosoma brucei* is a determinant of host pathology. *PLoS Negl Trop Dis* **3**: doi:10.1371/journal.pntd.0000557

Morrison LJ, McLellan S, Sweeney L, Chan CN, MacLeod A, Tait A, Turner MR. 2010. Role for parasite genetic diversity in differential host responses to *Trypanosoma brucei* infection. *Infect Immun* **78**: 1096-1108.

Moriyama EN, Powell JR. 1997. Synonymous substitution rates in *Drosophila*: Mitochondrial versus nuclear genes. *J Mol Evol* **45**: 378 – 391.

Ogden NH, Maarouf A, Barker IK, Bigras-Poulin M, Lindsay LR, Morshed MG, O'Callaghan CJ, Ramay F, Waltner-Toews D, Charron DF. 2006. Climate change and the potential for range expansion of the Lyme disease vector *Ixodes scapularis* in Canada. *Int J Parasit* **36**: 63 – 70.

Ostfeld RS. 2011. Lyme disease. The ecology of a complex system. Oxford University Press, Oxford, UK.

Pemberton JM, Slate J, Bancroft DR, Barrett JA. 1995, Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Mol Ecol* **4**: 249-252.

Primmer CR, Møller AP, Ellegren H. 2009. A wide-range survey of cross-species microsatellite amplification in birds. *Mol Ecol* **5**: 365 – 378.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Risenfeld CS, Schloss PD, Handelsman J. 2004. Metagenomics: Genomic analysis of microbial communities. *Annu Rev Genet* **38**: 525-552.

Samuel WM. 2004. White as a Ghost: Winter Ticks and Moose. Federation of Alberta Naturalists, Edmonton, Alberta, Canada.

Schlötterer C. 2000. Evolutionary dynamics of microsatellite DNA. *Chromosoma* **109**: 365-371.

Yuan DT. 2011. A metagenomic study of the tick midgut. [M.Sc. Thesis] University of Texas

Zane L, Bargelloni L, and Patarnello T. 2002. Strategies for microsatellite

isolation: A review. *Mol Ecol* **11**: 1-16.

APPENDIX A

Listed below are literature/sources of information from which an approximate distribution of the winter tick *Dermacentor albipictus* was estimated (Figure 1-1). Presence of the organism in a state or province was based on literature in which they were either reported present or collected as part of a tick surveillance survey and other research purposes. This is by no way a complete list of all literature available on the topic.

The references are listed according to states or provinces in the United States of America and Canada respectively. Some sources might be cited repeatedly if there were mentions of *Dermacentor albipictus* presence in multiple regions. Lack of official records or failure at finding records for certain provinces or states do not necessarily imply absence of the parasite in the region.

Canada

Samuel WM. 2004. White as a ghost: Winter ticks and Moose. The Federation of Alberta Naturalist, Edmonton, Alberta.

Wilkinson PR. 1967. The distribution of *Dermacentor* ticks in Canada in relation to bioclimatic zones. *Can J Zool* **45**: 517-537.

Alberta

Brown JH, Kohls GM. 1950. The ticks of Alberta with special reference to distribution. *Can J Res* **28**: 197-205.

Canadian Cooperative Wildlife Health Centre. 1999. Newsletter Vol. 6-2.

Cowan I. 1951. The diseases and parasites of big game mammals of western Canada. *Proc Ann Game Con* **5**:37-64

Flook DR, Stenton JE. 1969. Incidence and abundance of certain parasites in wapiti in the national parks of the Canadian Rockies. *Can J Zool* **47**: 795-803.

Keer GR, Holmes JC. 1966. Parasites of mountain goats in west central Alberta. *J Wildl Manage* **30**: 786-790.

Leighton FA. 2000. Winter tick in moose and other ungulates. http://www.ccwhc.ca/wildlife_health_topics/winter_tick/wintertick.php#canada. Assessed 29 August 2011.

Pybus M. 1999. Moose and ticks in Alberta: a die-off in 1998-99. Occas. Pap. No. 20, Fish.Wildl. Mgmt. Div., AB Environ. (available from Information Centre, Tel. 780 422 2074).

Samuel WM. 1989. Locations of Moose in Northwestern Canada with hair loss probably caused by the winter tick, *Dermcentor albipictus* (Acari: Ixodidea). *J Wildl Dis* **25**: 436-439.

Samuel WM, Welch DA. 1991. Winter ticks on moose and other ungulates: Factors influencing their population size. *Alces* **27**: 169-182.

Scoles GA. 2004. Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. *J Med Entomol* **41**: 277-286.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA, pp. 30-32.

British Columbia

Blood DA. 1963. Parasites from California Bighorn Sheep in southern British Columbia. *Can J Zool* **41**: 913-918.

Canadian Cooperative Wildlife Health Centre. 1999. Newsletter Vol. 6-2.

Flook DR, Stenton JE. 1969. Incidence and abundance of certain parasites in wapiti in the national parks of the Canadian Rockies. *Can J Zool* **47**: 795-803.

Humphrey FA, Gibbons RJ. 1942. Some observations on corynebacterial infections with particular reference to their occurrence in mule deer, *Odocoileus hemionus*, in British Columbia. *Can J Comp Med* **6**: 35-45.

Ritcey RW, Edwards RY. 1958. Parasites and diseases of the Wells Gray moose herd. *J Mammal* **39**: 139-145

Samuel WM. 1989. Locations of Moose in Northwestern Canada with hair loss probably caused by the winter tick, *Dermacentor albipictus* (Acari: Ixodidea). *J Wildl Dis* **25**: 436-439.

Samuel WM, Welch DA. 1991. Winter ticks on moose and other ungulates: Factors influencing their population size. *Alces* **27**: 169-182.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA, pp. 30-32.

Manitoba

Banfield AWF. 1949. An irruption of elk in Riding Mountain Park, Manitoba. *J Wildl Manage* **13**: 127-134.

Samuel WM, Welch DA. 1991. Winter ticks on moose and other ungulates: Factors influencing their population size. *Alces* **27**: 169-182.

New Brunswick

Bequaert JC. 1946**.** The ticks, or Ixodoidea, of the northeastern United States and eastern Canada. *Entomol Am* **25**: 73-232**.**

Canadian Cooperative Wildlife Health Centre. 1999. Newsletter Vol. 6-2.

Gregson JD. 1956. The Ixodoidae of Canada. Canada Department of Agriculture Entomology Division, Publication 930.

Newfoundland and Labrador

~ Not present in province~

Northwest Territories

Government of the Northwest Territories Department of Environment and Natural Resources. 2005. Winter Ticks. http://www.enr.gov.nt.ca/_live/pages/wpPages/Winter_ticks.aspx. Assessed 25 August 2011.

Kutz SJ, Jenkins EJ, Veitch AM, Ducrocq J, Polley L, Elkin B, Lair S. 2009 The Arctic as a model for anticipating, preventing,and mitigating climate change impacts on host-parasite interactions. *Vet Parasit* **163**: 217–228

Samuel WM. 1989. Locations of Moose in Northwestern Canada with hair loss probably caused by the winter tick, *Dermacentor albipictus* (Acari: Ixodidea). *J Wildl Dis* **25**: 436-439.

Nova Scotia

Benson DA. 1958. Moose "sickness" in Nova Scotia – I. *Can J Com Med* **22**: 244-248.

Bequaert JC. 1946**.** The ticks, or Ixodoidea, of the northeastern United States and eastern Canada. *Entomol Am* **25**: 73-232**.**

Gregson JD. 1956. The Ixodoidae of Canada. Canada Department of Agriculture Entomology Division, Publication 930.

Martella M,Yescott RE, Dodds DG. 1969. Some records for Ixodidae of Nova Scotia. *Can J Zool* **47**: 183-184**.**

Nova Scotia Department of Natural Resources. 2007. Recovery Plan for Moose (*Alces alces Americana*) in Mainland Nova Scotia.

Wright B. 1979. Mites, ticks, fleas and lice in the Nova Scotia Museum and Acadia University Museum Collections . *Proc Nova Scotian Inst Sci* **29**: 185-196.

Nunavut

~No record found~

Ontario

Addison EM, Johnson FJ, Fyvie A. 1979. *Dermacentor albipictus* on moose (*Alces alces*) in Ontario. *J Wildl Dis* **15**: 281-284.

Canadian Cooperative Wildlife Health Centre. 1999. Newsletter Vol. 6-2.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Whitlaw HA, Lankester MW. 1994. The co-occurrence of moose, white-tailed deer, and *Parelaphostrongylus tenuis* in Ontario. *Can J Zool* **72**: 819-825.

Prince Edward Island

~No record found~

(Note: It is believed that Prince Edward lacks the necessary ungulate populations to support any winter tick populations (pers. comm. Dr. W. Samuel 2011))

Quebec

Nguon S, Milord F, Trudel L, Ogden N, Lindsay R, Bouchard C, Fournier S. 2009. Étude épidémiologique sur les zoonoses transmises par les tiques dans le sud-ouest du Québec – 2008. Institut national de santé publique du Québec. Québec, Québec, Canada.

Vaillancourt P. 2011. Des orignaux morts par la tique d'hiver. http://www.lechodelatuque.com/Actualites/Actualites-regionales/2011-05-18/article-2516856/Des-orignaux-morts-par-la-tique-d%26rsquo%3Bhiver/1. Assessed 13 September 2011.

Wells E. 2010. La Tique d'hiver est présente en Gaspésie, comme au BSL! http://progresecho.canoe.ca/webapp/sitepages/content.asp?contentid=161423&id=331. Assessed 12 September 2011.

Saskatchewan

Cameron AE, Fulton JS. 1927. A local outbreak of the winter tick, *Dermacentor albipictus*, Pack. (Ixodoidea) in Saskatchewan. *Bull Entomol Res* **17**: 249-257.

Canadian Cooperative Wildlife Health Centre. 1999. Newsletter Vol. 6-2.

Yukon Territory

Leighton, F. A. 2000. Winter tick in moose and other ungulates. http://www.ccwhc.ca/wildlife_health_topics/winter_tick/wintertick.php#canada. Assessed 29 August 2011.

Yukon Department of Environment. 2010. Fish and Wildlife Branch Highlights 2009-2010. Yukon Department of Environment, Whitehorse, Yukon.

UNITED STATES OF AMERICA

Bishopp FC, Trembley H. 1945. Distribution and hosts of certain North American ticks. *J Parasitol* **31**:1–54.

Bishopp FC, Wood HP. 1913. The biology of some North American ticks of the genus *Dermacentor*. *Parasitol Camb* **6**: 153-187.

Cooley RA. 1938. The genera *Dermacentor* and *Otocentor* (Ixodidae) in the United States, with studies in variation. National Institute of Health Bulletin No. 171. US Government Printing Office, Washington, USA.

Alabama

Durden LA, Luckhart S, Mullen GR, Smith S. 1991. Tick infestations of white-tailed deer in Alabama. *J Wildl Dis* **27**: 606-614

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Luckhart S, Mullen GR, Durden LA, Wright JC. 1992. *Borrelia* sp. in ticks recovered from white-tailed deer in Alabama. *J Wildl Dis* **28**: 449-452.

Mullen GR, Luckhart S, Durden LA. 1990. Four tick species found on Alabama deer. *High Agri Res* **37**: 9

Smith JS. 1977. A survey of ticks infesting white-tailed deer in 12 southwestern states. [M.Sc. Thesis] University of Georgia

Alaska

~Not present in state~

(*NOTE: It had been shown that given the right conditions, it is possible for winter tick populations to establish in the state.)* [Zarnke RL, Samuel WM, Franzmann AW, Barrett R. 1990. Factors influencing the potential establishment of the winter tick (*Dermacentor albipictus*) in Alaska. *J Wildl Dis* **26**: 412-415]

Arizona

Arizona Veterinary Diagnostic Laboratory. 1997. Newsletter: Quarterly publication of the Arizona Veterinary Diagnostic Laboratory and Cooperation Extension. Volume 2, Issue 2. The University of Arizona, Tucson, Arizona, USA.

Neal BJ. 1959. A contribution on the life history of the collared peccary in Arizona. *Am Mid Nat* **61**: 177-190

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Arkansas

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Medl Entomol* **8**: 495-498

California

Furman DP, Loomis EC. 1984. Ticks of California (Acari: Ixodida). University of California Press, Berkeley, CA.

Jessup DA, Goff WL, Stiller D, Oliver MN, Bleich VC, Boyce WM. 1993. A retrospective serologic survey for *Anaplasma spp.* infection in three bighorn sheep (*Ovis canadensis*) populations in California. *J Wildl Dis* **29**: 547-554

Lane RS. 1996. Risk of human exposure to vector ticks (Acari: Ixodidae) in a heavily used recreational area in northern California. *Am J Trop Med Hyg* **55**：165-173.

Lane RS, Emmons RW, Dondero DV, Nelson BC. 1981. Ecology of tick-borne agents in California: I. Spotted fever group Rickettsiae. *Am J Trop Med Hyg* **30**：239-252

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Colorado

Eisen L, Ibarra-Juarez LA, Eisen RJ, Piesman J. 2008. Indicators for elevated risk of human exposure to host-seeking adults of the Rocky Mountain wood tick (*Dermacentor andersoni*) in Colorado. *J Vector Ecol* **33**: 117-128

Karns PD 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Connecticut

Anderson JF, Magnarelli LA. 1980. Vertebrate host relationships and distribution of Ixodid ticks (Acari: Ixodidae) in Connecticut, USA. *J Med Entomol* **17**: 314-323

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Magnarelli LA, Anderson JF, Apperson CS, Fish D, Johnson RC, Chappell WA. 1986. Spirochetes in ticks and antibodies to *Borrelia burgdorferi* in white-tailed deer from Connecticut, New York state, and North Carolina. *J Wildl Dis* **22**: 178-188

Main AJ, Sprance HE, Kloter KO, Brown SE. 1981. *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer (*Odocoileus virginianus*) in Connecticut. *J Med Entomol* **18**: 487-492

Wallis RC, Brown SE, Kloter KO, Main AJ Jr. 1978. Erythema chronicum migrans and Lyme arthritis: Field study of Ticks. *Am J Epidemiol* **108**: 322-327.

Delaware

~No records found~

Florida

Forrester DJ. 1992. Parasites and diseases of Wild mammals in Florida. University Press of Florida, Gainesville, Florida.

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Georgia

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J MedEntomol* **8**: 495-498

Lavender DR, Oliver JH. 1996. Ticks (Acari: Ixodidae) in Bulloch County, Georgia. *J Med Entomol* **33**: 224-231

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Wilson N, Baker WW. 1972. Ticks of Georgia (Acarina: Metastigmata). Bulletin of the Tall Timbers Research Station, Tallahassee, Florida, USA.

Hawaii

Garrett LE, Haramoto FH. 1967. A catalog of Hawaiian Acarina. *Proc Hawaiian Entomol Soc* **19**: 381-414.

Idaho

Brandborg SM. 1955. Life history and management of the mountain goat in Idaho. *Idaho Dept Fish and Game Wildl Bull* **2**: 142.

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Mills HB. 1937. A preliminary study of the Bighorn of Yellowstone National Park. *J Mammal* **18**: 205-212.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Illinois

Cortinas MR, Kitron U. 2006. County-level surveillance of white-tailed deer infestation by *Ixodes scapularis* and *Dermacentor albipictus* (Acari: Ixodidae) along the Illinois River. *J Med Entomol* **43**: 810-819

Illinois Department of Public Health Prevention and Control – Common ticks. http://www.idph.state.il.us/envhealth/pcommnticks.htm. Assessed 19 August 2011.

Indiana

Keefe LM, Moro MH, Vinasco J, Hill C, Wu CC, Raizman EA. 2009. The use of harvested white-tailed deer (*Odocoileus virginianus*) and geographic information system (GIS) methods to characterize distribution and locate spatial clusters of *Borrelia burgdorferi* and its vector *Ixodes scapularis* in Indiana. *Vector borne Zoo Dis* **9**: 671-680.

Pinger RR. 1999. Rocky Mountain spotted fever and Lyme disease surveillance program in Indiana: Final report 1999. http://www.in.gov/isdh/reports/disease/1999/app_b.htm. Assessed 19 August 2011.

Iowa

Lindgren M, Rowley WA, Thompson C, Gilchrist M. 2005. Geographic distribution of ticks (Acari: Ixodidae) in Iowa with emphasis on *Ixodes scapularis* and their infection with *Borrelia burgdorferi*. *Vector borne Zoo Dis* **5**: 219-226.

Kansas

Biology 625 Animal Parasitology - Distributional maps of various tick species in Kansas. http://www.kstate.edu/parasitology/625tutorials/tick04.html. Assessed 28 August 2011.

Brillhart DB. 1993. Sero-prevalence of Lyme disease antibodies in *Peromyscus leucopus*, *Peromyscus maniculatus* and *Odocoileus virginianus*, and distribution of potential tick vectors, in Kansas. [M.Sc. thesis] Kansas State University

Kentucky

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Taft SC, Miller MK, Wright SM. 2005. Distribution of Borreliae among ticks collected from eastern States. *Vector-borne Zoo Dis* **5**: 383-389.

Louisiana

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Maine

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Karkos T. 2011. Woodsmen, butcher say Maine moose and deer crawling with ticks. Sun Journal http://m.sunjournal.com/news/oxford-hills/2011/12/01/woodsmen-butchers-say-maine-moose-and-deer-crawling-ticks/1122280. Retrieved 2nd December 2011. [no official id]

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Rand PW, Lacombe EH, Dearborn R, Cahill B, Elias S, Lubelczyk CB, Beckett GA, Smith RP. 2007. Passive surveillance in Maine, an area emergent for tick-borne diseases. *J Med Entomol* **44**:1118-1129

Smith RP, Rand PW, Lacombe EH. 1990. Potential for Lyme Disease in Maine: Deer survey of distribution of *Ixodes dammini*, the tick vector. *Am J Public Health* **80**:333-335

Maryland

Amerasinghe FP, Breisch NL, Azad AF, Gimpel WF, Greco M, Niehardt K, Pagac B, Piesman J, Sandt J, Scott TW. 1992. Distribution, density, and Lyme disease spirochete infection in *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Maryland. *J Med Entomol* **29**:54–61.

Amerasinghe FP, Breisch NL, Neidhardt K, Pagac B, Scott TW. 1992. Distribution of the Winter Tick *Dermacentor albipictus* (Acari: Ixodidae) in Maryland. *Bull Soc Vector Ecol* **17**: 109-113

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Oliver JH, Magnarelli LA, Hutcheson HJ, Anderson JF. 1999. Ticks and antibodies to *Borrelia burgdorferi* from mammals at Cape Hatteras, NC and Assateague Island, MD and VA. *J Med Entomol* **36**: 578-587.

Massachusetts

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Michigan

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Lawrence WH, Hays KL., Graham SA. 1961. Ectoparasites of the beaver (*Castor Canadensis* Kuhl). Wildlife Disease. No. 12.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Walker ED, Stobierski MG, Poplar ML, Smith TW, Murphy AJ, Smith PC, Schmitt SM, Cooley TM, Kramer CM.1998. Geographic distribution of ticks (Acari: Ixodidae) in Michigan, with Emphasis on *Ixodes scapularis* and *Borrelia burgdorferi. J Med Entomol* **35**: 872-882

Minnesota

Baldridge GD, Scoles GA, Burkhardt NY, Schloeder B, Kurtti TJ, Munderloh UG. 2009. Transovarial transmission of *Francisella*-like endosymbionts and *Anaplasma phagocytophilum* variants in *Dermacentor albipictus* (Acari: Ixodidae). *J Med Entomol* **46**: 625-632

Edwards AJ, Schrage M, Lenarz M. 2004. Northeastern Minnesota moose management – A case study in cooperation. *Alces* **40**: 23-31

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Rogers LL. 1975. Parasites of black bears of the Lake Superior region. *J Wildl Dis* **11**: 189-192.

Gill JS, Johnson RC, Sinclair ML, Weisbrod AR.1993. Prevalence of the Lyme disease spirochete, *Borrelia burgdorferi*, in deer ticks (*Ixodes dammini*) collected from white-tailed deer (*Odocoileus virginianus*) in Saint Croix State Park, Minnesota. *J Wildl Dis* **29**: 64-72

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Mississippi

Demerais S, Jacobson HA, Guynn DC. 1987. Effects of season and area on ectoparasite of white-tailed deer (*Odocoileus virginianus*) in Mississippi. *J Wildl Dis* **23**: 261-266.

Goddard J. 2002. A ten-year study of tick biting in Mississippi: Implications for human disease transmission. *J Agromed* **8**: 25-32

Goddard J. 2006. An annotated list of the ticks (Ixodidae and Agarsidae) of Mississippi. *J Vector Ecol* **31**: 206-209

Goddard J, Norment BR. 1985. A guide to the ticks of Mississippi. Bulletin No. 935, Mississippi Agricultural and Forest Experiment Station, Mississippi State, Mississippi, USA.

Missouri

Kollars TM Jr, Durden LA, Masters EJ, Oliver JH. Jr. 1997. Some factors affecting infestation of white-tailed deer by blacklegged ticks and winter ticks (Acari:Ixodidae) in southeastern Missouri. *J Med Entomol* **34**: 372-375

Kollars TM, Oliver JH, Masters EJ, Kollars PG, Durden LA. 2000. Host utilization and seasonal occurrence of *Dermacentor* species (Acari: Ixodidae) in Missouri, USA. *Exp Appl Acarol* **24**: 631-643

Montana

Becklund WW, Senger CM. 1967. Parasites of *Ovis canadensis Canadensis* in Montana, with a checklist of the internal and external parasites of the Rocky Mountain Bighorn Sheep in North America. *J Parasitol* **53**: 157-165

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Mills HB. 1937. A preliminary study of the Bighorn of Yellowstone National Park. *J Mammal* **18**: 205-212.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Yunker CE, Keirans JE, Clifford CM, Easton ER. 1986. *Dermacentor* ticks (Acari: Ixodoidea: Ixodidae) of the New World: A scanning electron microscope atlas. *Proc Entomol Soc Wash* **88**: 609-627

Nebraska

Campbell JB. 2002. EC02-1550 Nebraska management guide for arthropod pests of livestock and horses. US Department of Agriculture and University of Nebraska, Nebraska, USA.

Campbell JB, Thomas GD. 1994. G94-1220 Controlling ticks. US Department of Agriculture and University of Nebraska, Nebraska, USA.

University of Nebraska – Lincoln Extension, Institute of Agriculture and Natural Resources. n. d. Controlling ticks. http://elkhorn.unl.edu/epublic/pages/publicationD.jsp?publicationId=500. Assessed 26 August 2011.

Nevada

Beck DE, Allred DM, Brinton EP. 1963. Ticks of the Nevada test site. Brigham Young University Science Bulletin, Biological Series 7.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

New Hampshire

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Musante AR. 2006. Characteristics and dynamics of a moose population in northern New Hampshire. [M.Sc. Thesis]. University of New Hampshire

Strickland J. 2011. New Hampshire: Shorter winters mean more ticks, pose big threat to moose. http://www.fws.gov/news/blog/index.cfm/2011/6/17/New-Hampshire-Shorter-Winters-Mean-More-Ticks-Pose-Big-Threat-to-Moose. Assessed 28 August 2011.

New Jersey

Schulze TL, Lakat MF, Bowen GS, Parkin WE, Shisler JK. 1984. *Ixodes dammimi* (Acari: Ixodidae) and other ixodid ticks collected from white-tailed deer *Odocoileus virginianus* in New Jersey USA. Geographical distribution and its relation to selected environmental and physical factors. *J Med Entomol* **21**: 741-749

Taft SC, Miller MK, Wright SM. 2005. Distribution of Borreliae among ticks collected from eastern States. *Vector borne Zoo Dis* **5**: 383-389.

New Mexico

Allen RW. 1955. Parasites of mountain sheep in New Mexico, with new host records. *J Parasitol* **41**: 583-587

Meleney WP. 1975. Arthropod parasites of the collared peccary, *Tayassu tajacu* (Artiodactyla: Tayassuidae), from New Mexico. *J Parasitol* **61**: 530-534

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Wilson GI. 1969. Some parasites of elk in New Mexico. *Bull Wildl Dis Ass* **5**: 1969

New York

Arthur DR. 1960. Ticks: A monograph of the Ixodidae part V on the genera *Dermacentor, Anocentor, Cosmiomma, Boophilus*, and *Margaropus*. Cambridge University Press, UK.

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Nuttall GHF, Cooper WF, Warburton C, Robinson LE. 1908. Ticks, a monograph of the Ixodoidea Volume 2 part 2. University Press, Harvard University, Cambridge, Massachusetts, USA.

Taft SC, Miller MK, Wright SM. 2005. Distribution of Borreliae among ticks collected from eastern States. *Vector borne Zoo Dis* **5**: 383-389.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United

States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

North Carolina

Apperson CS, Levine JF, Nicholson WL. 1990. Geographic occurrence of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) infesting white-tailed deer in North Carolina. *J Wildl Dis* **26**: 550-553

Reeves WK, Durden LA, Ritzi CM, Beckham KR, Super PE, Oconnor BM. 2007. Ectoparasites and other ectosymbiontic arthropods of vertebrates in the Great Smoky Mountains National Park, USA. *Zootaxa* **1392**:31-68

North Dakota

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

The National Lyme Disease Memorial Park Project. n. d. State statistics: North Dakota http://www.lymememorial.org/State_Stats/State_Statistics_North_Dakota.htm. Assessed 29 August 2011.

Ohio

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Ohio Department of Health. 2010. Tick borne diseases: Ohio summary 2010. Bureau of Infectious Disease, Ohio Department of Health, Ohio, USA.

Oklahoma

Arthur DR. 1960. Ticks: A monograph of the Ixodidae part V on the genera *Dermacentor, Anocentor, Cosmiomma, Boophilus*, and *Margaropus*. Cambridge University Press, UK.

Ewing SA, Panciera RJ, Kocan KM, Ge NL, Welch RD, Olson RW, Barker RW, Rice LE. 1997. A winter outbreak of anaplasmosis in a nonendemic area of Oklahoma: a possible role for *Dermacentor albipictus*. *J Vet Diag Invest* **9**: 206-208

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Kocan AA. n.d. Ticks and tick-transmitted diseases in Oklahoma. http://instructions.cvhs.okstate.edu/kocan/ticks/tickOK.htm. Assessed 27 August 2011.

Kocan AA, Mukolwe SW, Murphy GL, Barker RW, Kocan KM. 1992. Isolation of *Borrelia burgdorferi* (Spirochaetales: Spirochaetaceae) from *Ixodes scapularis* and *Dermacentor albipictus* ticks (Acari: Ixodidae) in Oklahoma. *J Med Entomol* **29**: 630-633.

Nuttall GHF, Cooper WF, Warburton C, Robinson LE. 1908. Ticks, a monograph of the Ixodoidea Volume 2 part 2. University Press, Harvard University, Cambridge, Massachusetts, USA.

Patrick CD, Hair JA. 1975. Ecological observations on *Dermacentor albipictus* (Packard) in eastern Oklahoma (Acarina: Ixodidae). *J Med Entomol* **12**: 393-394

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Oregon

Parsons GL, Rossignol PA. 1989. Identifying adult hard ticks commonly found on humans in Oregon. Oregon State University Extension Service, Corvallis, Oregon, USA.

Peterson KJ, Kistner TP, Davis HE. 1973. Epizootiologic studies of Anaplasmosis in Oregon mule deer. *J Wildl Dis* **9**: 314-319.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Pennsylvania

Arthur DR. 1960. Ticks: A monograph of the Ixodidae part V on the genera *Dermacentor, Anocentor, Cosmiomma, Boophilus*, and *Margaropus*. Cambridge University Press, UK.

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Nuttall GHF, Cooper WF, Warburton C, Robinson LE. 1908. Ticks, a monograph of the Ixodoidea Volume 2 part 2. University Press, Harvard University, Cambridge, Massachusetts, USA.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Rhode Island

Hyland KE, Mathewson JA. 1961. The ectoparasites of Rhode Island Mammals I. The Ixodid Tick Fauna. Wildlife Disease. No. 11

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

South Carolina

Clark KL, Oliver JH Jr, McKechnie DB, Williams DC. 1998. Distribution, abundance, and seasonal activities of ticks collected from rodents and vegetation in South Carolina. *J Vector Ecol* **23**: 89-105

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Williams DC, Wills W, Durden LA, Gray EW. 1999. Ticks of South Carolina (Acari: Ixodidae). *J Vector Ecol* **24**: 224-232

South Dakota

Brokaw C. 2009. Ticks, other factors tied to cattle, deer deaths. http://www.lymememorial.org/State_Articles/Articles_SD.htm. Assessed 29 August 2011.

Daly R. 2009. Early spring cow losses in southwest South Dakota: Clinical presentations and investigations. *Animal Health Matters* **Volume 12**, Veterinary Science Department, South Dakota State University, South Dakota, USA.

Tennessee

Arthur DR. 1960. Ticks: A monograph of the Ixodidae part V on the genera *Dermacentor, Anocentor, Cosmiomma, Boophilus*, and *Margaropus*. Cambridge University Press, UK.

Clifford CM, Anastos G, Elbl A. 1961. The larval Ixodid ticks of the eastern United States (Acarina-Ixodidae). *Misc Pub Entomol Soc Am* **2**:215-237.

Cooney JC, Burgdofer W. 1974. Zoonotic potential (Rocky Mountain spotted fever and tularemia) in the Tennessee Valley region. I. Ecologic studies of ticks infesting mammals in Land Between the Lakes. *Am J Trop Med Hyg* **23**: 99-108.

Durden LA, Kollars TM Jr. 1992. An annotated list of the ticks (Acari: Ixodoidea) of Tennessee, with records of four exotic species for the United States. *Bull Soc Vector Ecol* **17**: 125-131

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Nuttall GHF, Cooper WF, Warburton C, Robinson LE. 1908. Ticks, a monograph of the Ixodoidea Volume 2 part 2. University Press, Harvard University, Cambridge, Massachusetts, USA.

Shariat BM, Freimund JA, Wright SM, Murphree CS, Thomas JT. 2007. *Borrelia* infection rates in winter ticks (*Dermacentor albipictus*) removed from white-tailed deer (*Odocoileus virginanus*) in Cheatham County, Tennessee. *J Tennessee Acad Sci* **82**: 57-61

Texas

Arthur DR. 1960. Ticks: A monograph of the Ixodidae part V on the genera *Dermacentor, Anocentor, Cosmiomma, Boophilus*, and *Margaropus*. Cambridge University Press, UK.

Drummond RO. 1967. Seasonal activity of ticks (Acarina: Megastigmata) on cattle in southwestern Texas. *Ann Entomol Soc Am* **60**: 439-447

Hailey TL, Thomas JW, Robinson RM. 1966. Pronghorn Die-off in Trans-Pecos Texas. *J Wildl Manage* **30**：488-496。

Holman PJ, Craig TM, Crider DLD, Petrini KR, Rhyan J, Gale, G. 1994. Culture Isolation and partial characterization of a *Babesia sp.* From a North American elk (*Cervus elaphus*). *J Wildl Dis* **30**: 460-465

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Nuttall GHF, Cooper WF, Warburton C, Robinson, L. E. 1908. Ticks, a monograph of the Ixodoidea Volume 2 part 2. University Press, Harvard University, Cambridge, Massachusetts, USA.

Osborn DA. 1990. Physical condition evaluation of axis, fallow, sika, and white-tailed deer in central Texas. [M.Sc. thesis] Texas Tech University.

Richardson ML, Demaraia S. 1992.Parasites and condition of coexisting populations of white-tailed and exotic deer in south-central Texas. *J Wildl Dis* **28**: 485-489

Scoles GA. 2004. Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. *J Med Entomol* **41**: 277-286.

Teel PD, Fuchs TW, Huston JE, Longnecker MT, Pickel SL. 1990. Effects of sequential infestation of *Dermacentor albipictus* and *Amblyomma americanum* (Acari: Ixodidae) on overwintering beef cows in west-central Texas. *J Med Entomol* **27**:632-641

Texas A&M University Tick Research Laboratory. n. d. Map of Texas depicting tick collections by county. http://ticsys.tamu.edu/map_fi.htm. Assessed 27 August 2011.

Walker ML, Becklund, W. W. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Utah

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Vermont

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Virginia

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Oliver JH, Magnarelli LA, Hutcheson HJ, Anderson JF. 1999. Ticks and antibodies to *Borrelia burgdorferi* from mammals at Cape Hatteras, NC and Assateague Island, MD and VA. *J Med Entomol* **36**: 578-587.

Taft SC, Miller MK, Wright SM. 2005. Distribution of Borreliae among ticks collected from eastern States. *Vector borne Zoo Dis* **5**: 383-389.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Washington

Foreyt WJ, Leathers CW. 1986. Traumatic reticulopericarditis in a mule deer. *J Wildl Dis* **22**: 446.

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

West Virginia

West Virginia University. n.d. West Virginia Tick Information. http://www.wvu.edu/~agexten/ipm/insects/2tick.htm . Assessed 26 August 2011.

Wisconsin

Arthur DR. 1960. Ticks: A monograph of the Ixodidae part V on the genera *Dermacentor, Anocentor, Cosmiomma, Boophilus*, and *Margaropus*. Cambridge University Press, UK.

Davis JP, Schell WL, Amundson TE, Godsey MS, Spielman A, Burgdorfer W, Barour AG, Laventure M, Kaslow RA. 1984. Lyme disease in Wisconsin: Epidemiologic, clinical, serologic, and entomologic findings. *Yale J Biol Med* **57**: 685-696

French JB, Schell WL, Kazmierczak JJ, Davis JP. 1992. Changes in population density of *Ixodes dammini* (Acari: Ixodidae) in Wisconsis. *J Med Entomol* **29**: 723-728

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Kellogg FE, Kistner TP, Strickland RK, Gerrish RR. 1971. Arthropod parasites collected from white-tailed deer. *J Med Entomol* **8**: 495-498

Manville AM. 1978. Ecto- and endoparasites of the black bear in northern Wisconsin. *J Wildl Dis* **14**: 97-101

Nuttall GHF, Cooper WF, Warburton C, Robinson LE. 1908. Ticks, a monograph of the Ixodoidea Volume 2 part 2. University Press, Harvard University, Cambridge, Massachusetts, USA.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

Wyoming

Karns PD. 2007. Population distribution, density and trends. In: Ecology and Management of the North American Moose, Second Edition (Eds. A.W. Franzmann and C.C. Schwartz) A Wildlife Management Institute Book, University Press of Colorado, Boulder, CO, USA. pp.125-139

Mills HB. 1937. A preliminary study of the Bighorn of Yellowstone National Park. *J Mammal* **18**: 205-212.

Samuel WM, Welch DA, Smith BL. 1991. Ectoparasites from elk (*Cervus elaphus nelsoni*) from Wyoming. *J Wildl Dis* **27**: 446-451.

Walker ML, Becklund WW. 1970. Index-catalogue of Medical and Veterinary Zoology: Special publication No. I Checklist of the internal and external parasites of deer, *Odocoileus hemionus* and *O. virginianus*, in the United States and Canada. U.S. Government Printing Office, Washington, USA. pp. 30-32.

BIOGRAPHY

As a Singaporean city-girl, born and raised in the concrete forest of high-rise apartments, shopping malls and office buildings, I hated and feared all forms of insects and arachnids. I shuddered at the thought of having insects in the vicinity of my person and would refuse to touch any pictures that bore an image of an arthropod. I never once entertained the thought of developing any interest in entomology or acarology, much less making the most disgusting and creepiest member of them all, the blood-sucking ticks, the main focus of my M.Sc. thesis.

I spend most of my formative years schooling in Singapore and attended pre-University level education at Hwa Chong Institution before immigrating to Canada to begin my undergraduate studies at the University of Alberta. My first year as an undergraduate was a carefree, fun and wonderful experience. Then came second year when I had to take an Entomology course as part of my degree requirements. Given my fear of anything with more than two pairs of legs that are not considered seafood, you can imagine the trepidation with which I first attended Dr. Felix Sperling's Introductory Entomology lecture. But as I continued forcing myself to attend one lecture after the other, I began to appreciate the complexity of arthropod body forms and gradually started to see more than just creepy freaks of nature when looking at these organisms. The introductory course perked my interest to such an extent that I approached Dr. Sperling at the end of the semester to discuss the probability of doing an NSERC summer undergraduate project under his tutelage. Thus began my foray into the fascinating, if a little strange, world of entomology, much to my parents' shocked dismay and my brother's unending horror (I like to think they have since grown accustomed to this strange twist in my life).

I have been a member of the Sperling lab since 2007, and have worked on several fascinating projects that allowed me to explore my growing interest in molecular genetics and entomology. I started out studying the population structure of *Halobates sericeus* on the Pacific Ocean in the summer of 2007, before moving

on to work on *Dermacentor albipictus* genetic diversity for my undergraduate project (2008-2009). Said undergraduate project has subsequently snowballed into my current Master's thesis.

I also spent two summers working as a technician assistant at the City of Edmonton Environmental Service Laboratory (formerly under the Department of Asset Management and Public Work). There, I met a wonderful group of people with the most devious sense of humour and deep appreciation for entomology. Their passion for their work further spurred me toward my own studies and research.

These past few years spent in the Sperling lab and with my friends at the City of Edmonton have been a wonderful learning experience. I will carry the memories of my time here as I make my way through life.