A MORPHOLOGICAL COMPARISON OF TWO CANADIAN POPULATIONS OF THE PINK STRIPED OAKWORM (*Anisota virginiensis* Drury) (LEPIDOPTERA: SATURNIIDAE)

by
Claire B. Hensrud

FACULTY OF NATURAL RESOURCES MANAGEMENT
LAKEHEAD UNIVERSITY
THUNDER BAY, ONTARIO

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Dr. Don Henne	Ryan Wilkie
Major Advisor	Second Reader

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ABSTRACT

Keywords: *Anisota virginiensis*, *Anisota*, morphology, pink-striped oakworm, wingspan, body length

This thesis involves a morphological and physiological comparison of two Canadian populations (Manitoba and Nova Scotia), and several USA populations of adult pink-striped oakworm (*Anisota virginiensis*) moths. It was hypothesized that there would be no significant differences in morphological characteristics of these adult moths (i.e., body length, wingspan, and colouration). Adult moth body lengths and wingspans were measured. By using a series of t-tests none of the populations were deemed to be significantly different. However there appears to be a modest trend towards smaller adult moths from northern and western parts of the range of this moth. Additionally, adult wing colouration was quite different amongst populations. Further research using mDNA procedures may indicate that certain populations of the pink-striped oakworm are new species.

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I would like to thank Dr. Don Henne for allowing me the opportunity to work on this project with him, and for all of his assistance and guidance throughout this process. I would also like to thank Ryan Wilkie for taking the time to be my second reader, my parents, Ole and Candi, and my fiancé Derian for always motivating me to do my best and helping me achieve my goals, as well as my dog Onni for the countless hours of company while I completed this study.

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INTRODUCTION

The pink-striped oakworm, *Anisota virginiensis* (Drury), is a member of the class Insecta, order Lepidoptera, and family Saturniidae (Burke and Peigler 2009). Oakworms are a term commonly used to refer to caterpillars of the genus *Anisota*. The genus *Anisota* includes ten known species that occur in the eastern portion of North America and six other species occurring in the southwest of North America and in Mexico (Burke and Peigler 2009).

Oakworms can be found throughout central and eastern North America in green spaces such as forests, yards, and parks. As the common name implies, they are known to occasionally cause severe oak (*Quercus spp*.) defoliation (Burke and Peigler 2009). From time to time, caterpillars of some species of *Anisota* can become pests and severe infestations amongst oak species can occur. *A. virginiensis* typically does not become abundant enough to become a pest, although it has caused severe defoliation of oaks within Manitoba, Ontario, and Quebec (Henne 2004).

The genus *Anisota* includes brownish coloured moths that vary from small to medium in size. There is a white spot on the forewing and sexual dimorphism is typically strong within the species (Riotte and Peigler 1980). *A. virginiensis* is a member of the *A. pellucida* group, which includes *A. virginiensis*, *A. pellucida*, and *A. discolor*, but some authors consider all three species to be synonymous with *A. virginiensis* (Tuskes et al. 1996). This group is determined through larval characteristics and adult moth habitats as well as the geographical distributions of the component species. Other groups include *A. stigma, A. senatoria*, and the Mexican groups (Riotte and Peigler 1980).

Anisota virginiensis caterpillars are mostly gray in colour and are covered with numerous white specks, and several rows of pink stripes run longitudinally down its body (Ferguson 1971). The moths of *A. virginiensis* are a pinkish-purplish colour. The males are diurnal, where the females are nocturnal (Ferguson 1971).

In this study, with the assistance of Dr. Don Henne, I will look at two widely separated Canadian populations of *A. virginiensis*; one from Manitoba, Canada and the other from Nova Scotia, Canada. These two populations will be compared morphologically in order to determine whether or not these are in fact two different species rather than one variable species. The population samples were collected by Dr. Henne in June of 2019 from both Belair Resort, Manitoba and Mount Uniake (Hants County), Nova Scotia. The caterpillars from Manitoba were reared from eggs collected from Bur Oak (*Quercus macrocarpa*), while eggs from Nova Scotia were obtained from a wild female collected in June of 2019. The adults of both populations emerged in the laboratory during early June of 2020. A number of fifth-instar caterpillars from the two populations were put into 95% ethanol and frozen for later DNA analysis.

The objective of my thesis is to examine two widely separated Canadian populations of *A. virginiensis*, as well as several USA populations from Dr. Henne's personal collection. This will be done through a series of body and wingspan measurements.

It is hypothesized that there is a difference in the phenotypic traits between the Manitoba and Nova Scotia populations of *A. virginiensis*.

LITERATURE REVIEW

THE GENUS *Anisota*

The genus *Anisota* is member of the family Saturniidae. The Saturniidae encompass some of the largest and showiest moths in North America (Tuskes et al. 1996). There are approximately 1200 species and more than 125 genera of Saturniidae worldwide, but only 18 genera and about 70 species occur north of Mexico (Tuskes et al. 1996), with one genus being *Anisota*. *Anisota* encompasses the smallest members of Saturniidae in North America (Ferguson 1971). The species within the genus *Anisota* have a white discal spot on their forewing, as well as a prominent apical spine on their front legs (Ferguson 1971). The caterpillars of *Anisota* are commonly known as oakworms. These caterpillars can be found throughout central and eastern North America in green spaces such as forests, yards and parks (Burke and Peigler 2009). On occasion, species of this group can become pests. Examples such as the yellow-striped oakworm (*A. peigleri* Riotte) and orange-striped oakworm (*A. senatoria*) are common pests in the United States (Henne 2004).

ECOLOGY/BIOLOGY OF THE PINK-STRIPED OAKWORM (*Anisota virginiensis*)

Female moths of *Anisota virginiensis* are nocturnal and are attracted to lights, whereas the male moths of most species are also partly diurnal and also fly during the day (Ferguson 1971).

Sexual dimorphism is very apparent in *A. virginiensis* (Figure 1). The males have a well-developed hyaline space on their forewing and a more rounded hindwing with a pinkish/ purplish red colour. The hindwing also has a straight outer margin. The darker wing colouration allows the moths to absorb the sunlight and raise their body temperature (Ellers and Boggs 2002). The females are a pinkish colour with no dark spots, and their forewings are longer and narrower. At the end of the foretibia both sexes have a short and obtuse spine (Ferguson 1971).

Figure 1. *Anisota virginiensis* moths from Nova Scotia. Male (left) and female (right). Source: Claire Hensrud.

Figure 2. *Anisota virginiensis* caterpillars from Nova Scotia (left) and Manitoba (right) in a lab setting. Source: Don Henne.

Anisota virginiensis caterpillars are gray with white specks (Figure 2). They have pink subdorsal and spiracular stipes. They primarily feed on oak species such as red oak (*Quercus rubra* Michx.) and bur oak (*Quercus macrocarpa* Michx.) (Ferguson 1971). The eggs of *A. virginiensis* are laid in clusters and consist of approximately 25-30 eggs (Henne 2004). Eggs of *Anisota* are known to be flat and oval-like and are laid on leaves of hosts (Riotte and Peigler 1980).

The adult moths of *A. virginiensis* mate using sex pheromones. By releasing sex pheromones, the female moths prompt the males to begin their search for them (Mason and Baruzzi 2019). In the late morning mating begins, and mating pairs stay joined together for the rest of the day. Once the day is over females set out to find a suitable place to lay their eggs. They typically target oak species (*Quercus spp*.) to lay their eggs on the underside of leaves (Mason and Baruzzi 2019). In a study done by Mason and Baruzzi (2019) it was discovered that the "host plant's condition is a critical aspect of the pink-striped oakworm moth life cycle" because the emerging larvae as they are their primary source of food (Mason and Baruzzi 2019). *A. virginiensis* larvae live in clusters known as colonies. The last instars will consume the entire leaf but leave the midvein behind (Henne 2004).

Populations of *Anisota virginiensis* typically do not exceed what would be normal population sizes. Because their populations do not normally reach outbreak status, they are not considered to be a pest (Henne 2004). Although it is not common, *A. virginiensis* populations have caused major defoliation of oaks in Manitoba, Ontario and Quebec on *Q. macrocarpa* (Henne 2004).

PREDATION AND PARASITISM OF *ANISOTA*

The members of the genus *Anisota* are attacked by many predators. Being the conspicuous caterpillars they are, they make an easy meal. Predators include, but are not limited to, spiders, wasps, birds, moles, mice, shrews and various pathogens (Serrano and Foltz 2004). The oakworms do not blend in with their defoliated hosts as they have aposematic colouration (Henne 2004). It was discovered by Henne (2004) that the fifth-instar larvae of *A. virginiensis* at Belair, Manitoba were experiencing parasitism by the fly family Tachinidae. Their approximately 1 mm ovoid eggs were laid onto the integument of the larvae (Henne 2004). Within the Tachinidae family, species such as *Houghia sternalis*, *Lespesia anisotae*, and *Winthemia datanae* all targeted *A. virginiensis* in Manitoba. In the Ichneumonidae family parasitic wasps also targeted *A. virginiensis*, species include *Habronyx magniceps* and *Hyposoter fugitivus* (Henne 2004). The orange striped oakworm is an example of a species, similar to the pink-striped oakworm, that is attacked by parasitoids (Coffelt and Schultz 1993).

ECOLOGY OF BUR OAK (*Quercus macrocarpa*) AND RED OAK (*Quercus rubra*)

Bur oak (*Quercus macrocarpa* (Michx.)) is a member of the Beech Family, Fagaceae. *Q. macrocarpa* typically grows to be a large tree reaching around 24 meters in height on average with a trunk of up to 90 cm in diameter (Row et al. 2012). Bur oak is deciduous and produces wood that is very hard and heavy. Leaves of *Q. macrocarpa* are typically alternate, ovate and lobed and appear dark green on the surface and green gray on its underside (Row et al. 2012). *Q. macrocarpa* is the most common native white oak in North America (Farrar 2017).

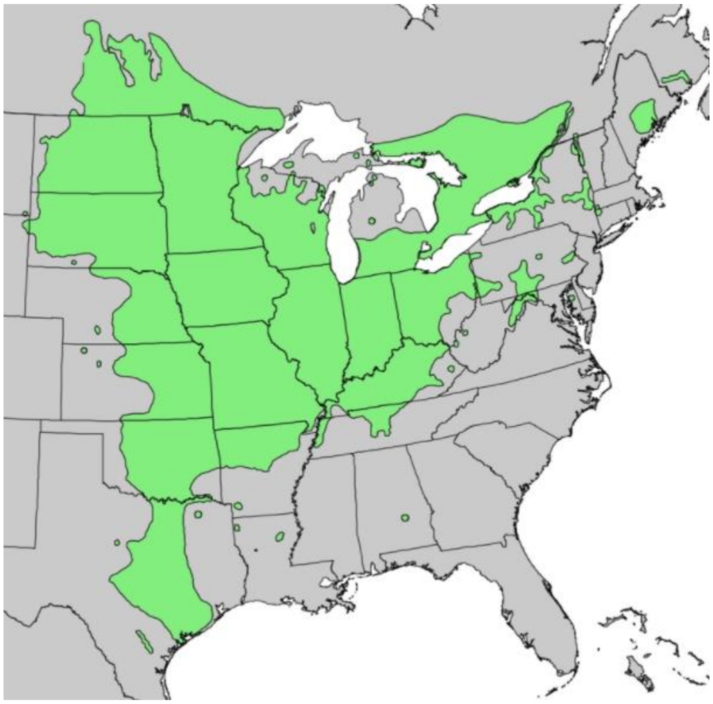

Figure 3. Range map of bur oak (*Quercus macrocarpa).* Source: U.S. Geological Survey by Elbert Little

The range of bur oak (Figure 3) is from Saskatchewan all the way to New Brunswick within Canada (Farrar 2017), as well as most northeastern and southern states (Little 1999). The tree produces cup-like acorns. *Q. macrocarpa* tends to grow best in areas with rich and deep soil, although it also grows in areas with limestone, and shallow soils over bedrock. It is a drought tolerant and fairly shade tolerant species (Farrar 2017). The *A. virginiensis* range overlaps those of bur oak and red oak.

Red oak (*Quercus rubra* (L.)) has also been found as a host for *A. Virginiensis* (Ferguson 1971). Red oak can grow up to 30 meters tall and as wide as 90 cm (OMNR). The leaves can be up to 20 cm long and consists of 7-9 lobes with bristled tips (Farrar 2017). They grow hairless, reddish-brown, cup shaped acorns. Once mature, the bark of red oak is deeply grooved and appears pale gray. Red oak prefers areas with a lot of sun light and room to grow (Farrar 2017).

Within Canada red oak can be found (Figure 4) around Lake Superior all the way to Nova Scotia (Farrar 2017). It also grows in the northeastern United States as far south as Alabama (Little 1971).

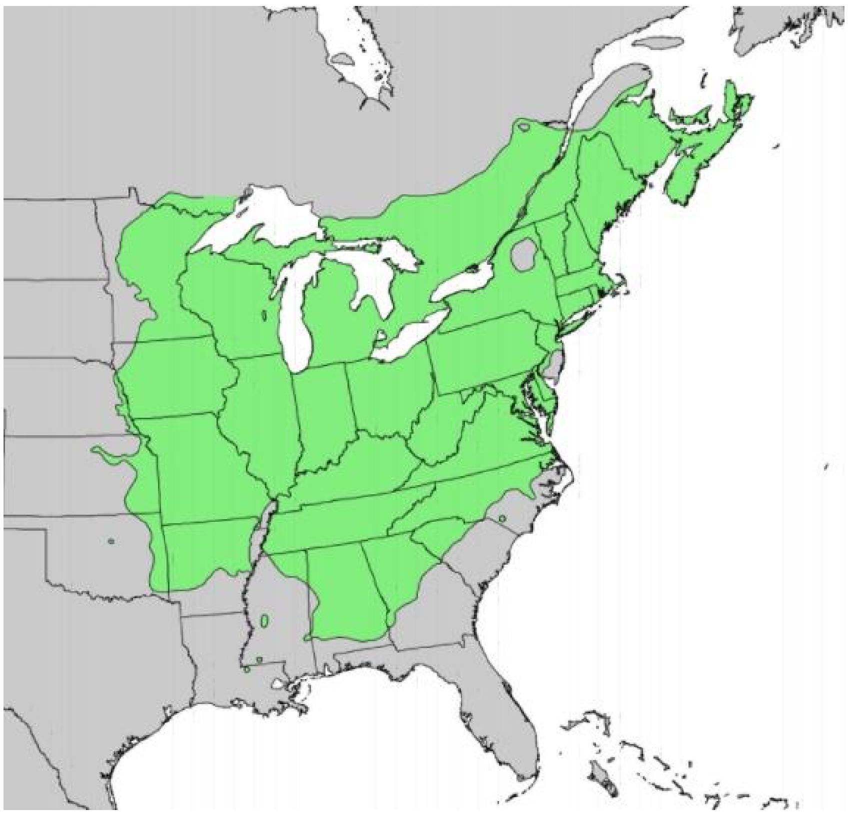

Figure 4. Range of red oak (*Quercus rubra*). Source: U.S. Geological Survey by Elbert Little.

GEOGRAPHICAL RANGE OF THE GENUS *Anisota*

Within eastern North America there are 10 known species and approximately six other species in the southwestern portion of North America (Burke and Peigler 2009). Species of *Anisota* can also be found throughout the majority of Mexico, into Central America (Burke and Peigler 2009).

Anisota virginiensis is commonly found in the central and eastern portion of southern Canada and south into the United States (Figure 5). It occurs from southeastern Manitoba to Nova Scotia south to Florida and Texas (Ferguson 1971). Within this range they can be found wherever oak species, their primary host, occur.

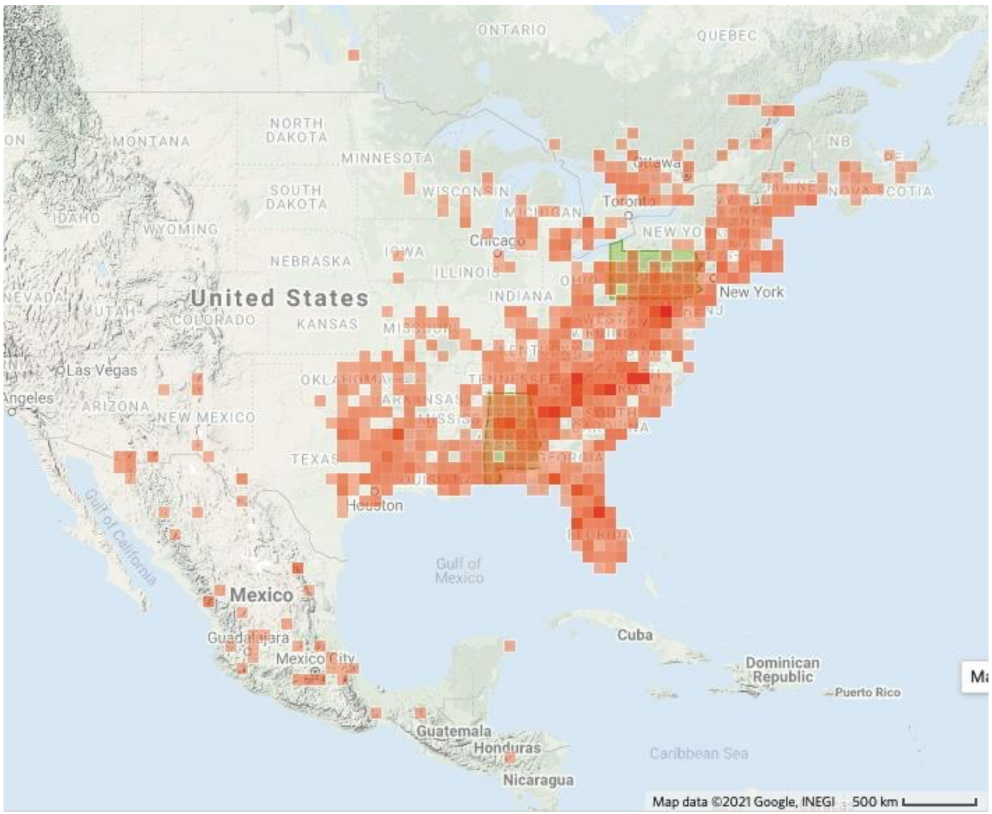

Figure 5. Range map of genus *Anisota.* Source: inaturalist.com

Table 1. List of *Anisota* species and their distribution. Source: Burke and Peigler (2009).

Species	Distribution
A. assimilis (Druce)	Mexico (Sierra Madre Occidental)
A. consularis Dyar	Southeastern USA
A. discolor Ferguson	Eastern Texas, Oklahoma
A. dissimilis (Boisduval)	Mexico
A. fuscosa Ferguson	Eastern Texas, western Louisiana
A. leucostygma (Boisduval)	Mexico (Oaxaca, Tamaulipas)
A. manitobensis McDunnough	Southern Manitoba, Wisconsin
A. oslari Rothschild	Southwestern USA
A. peigleri Riotte	Southeastern USA
A. pellucida (J.E. Smith)	Southeastern USA
A. senatoria (J.E. Smith)	Eastern North America
A. stigma (Fabricius)	Eastern North America
A. virginiensis (Drury)	Eastern North America

SPECIES RELATED TO *A. Virginiensis*

Anisota virginiensis is not a well-researched species and therefore the literature on this species is lacking. There are, however, other species related to *A. virginiensis* that have helped to provide a better understanding of the genus and its species.

The Manitoba oakworm, *Anisota manitobensis* (McD.), is a geographically restricted species that can be found from southern Manitoba to northern Minnesota and northeastern North Dakota (Henne 2002). The species is of special concern as there have been no individuals detected since 2000 (COSEWIC 2019). The male moths of *A. manitobensis* are a brownish-orange colour and females are a darker pink colour. The larvae are brown-black and have light stripes. Like most oakworms, they also have thoracic horns and spines (COSEWIC 2019). *A. manitobensis* largely depends on *Q. macrocarpa*. This host tree species is used by females to lay eggs and larvae feed on (COSEWIC 2019).

The orange-striped oakworm, *Anisota senatoria*, is another species related to *A. virginiensis*. *A. senatoria* can be found as far north as southern Ontario, south to the southern and midwestern United States (Coffelt 1992). The adults emerge from the pupae between June and July where they then mate on the ground or in trees (Coffelt and Schultz 1993). The eggs are yellow in colour and oviposited on different parts of the host tree during the month of July. *A. senatoria* prefers oak species (*Quercus sp*.) but other species such as maple (*Acer sp.*) and birch (*Betula sp*.) are targeted as well (Coffelt and Schultz 1993). *A. senatoria* is at times considered to be a pest as it has attacked and defoliated many tree stands throughout its range (Coffelt 1992).

MATERIALS AND METHODS

The samples of *Anisota virginiensis* used in this study were collected by Dr. Don Henne. The caterpillars (Figure 2) were reared from eggs collected from bur oak at Belair, Manitoba (Figure 6) during June 2019 at the approximate coordinates of 50° 37′ 19′′ 96° 34′ 50′′ W. Eggs of Nova Scotia *A. virginiensis* were sent to Dr. Henne from a colleague in Nova Scotia. The Nova Scotia population originated from a female captured at Mount Uniake (Hants County), Nova Scotia (Figure 7) at the approximate coordinates of 44° 52′ 49′′ N 63° 48′ 22′′ W. Caterpillars (Figure 2) from both populations were reared separately on Bur oak (*Quercus macrocarpa*) in the laboratory at Lakehead University in Thunder Bay during July and August of 2019.

It was noted by Dr. Henne that the mature (fifth-instar) caterpillars from these two populations were quite different in morphology and coloration and may represent two distinct species. After caterpillars completed feeding, they transformed into pupae and these were overwintered in a refrigerator in the laboratory until the following May. The adult moths emerged in the laboratory in early June of 2020. For future DNA analysis a few fifth-instar caterpillars from both populations were immersed in 95% ethanol and stored in a freezer.

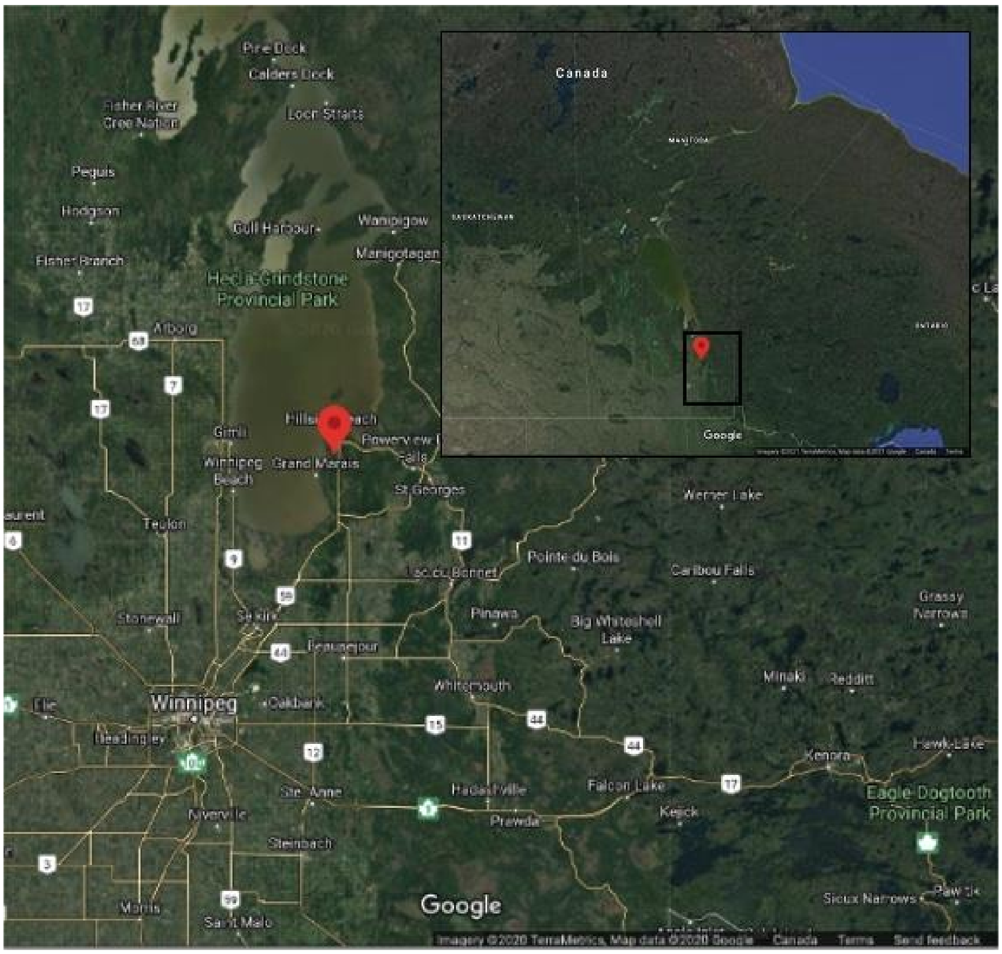

Figure 6. Map showing location of Belair, Manitoba. Source: Google Maps.

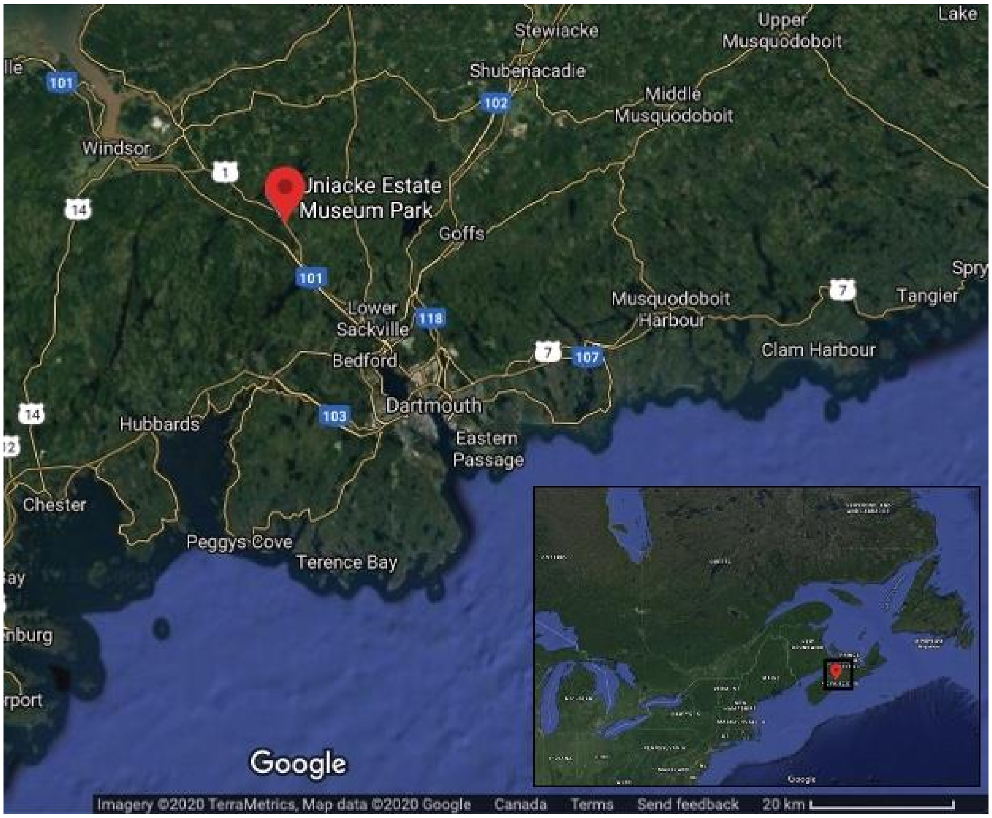

Figure 7. Map showing location of Mount Uniake, Nova Scotia. Source: Google Maps.

To determine if these two Canadian populations of *A. virginiensis* are indeed two different species, a series of simple morphological testing will be completed. Morphological tests include measuring the wing spans of the moths, in millimetres (mm), visually analyzing the differences between the males and females in the different populations and looking at differences between wing patterns and colours.

Once the adults emerged in the laboratory, Dr. Henne froze them and later dried and mounted each moth. Moths were then measured individually for both body length and wingspan. Measurements were done in mm using a Starrett caliper. Measurements were then organized into a series of tables. The wingspan and body length measurements were entered into a spreadsheet in order to perform a t-test to determine if there was a significant difference in these morphological measurements between the females and males from these two populations. Two-tailed, unequal variances t-tests were done in Microsoft Excel using an alpha significance value of 0.025.

RESULTS

The adult moth wingspans and body lengths varied within and between the populations and also between sexes within the two populations. Table 2 shows the measurements of the wingspans and body lengths from a sample of females of the Manitoba and Nova Scotia populations. By examining the table, one is able to see that the means for both body length and wingspan are higher in the Nova Scotia population then the Manitoba population.

Table 2. Wingspans and body lengths (in mm) of adult female *A. virginiensis* from the Manitoba and Nova Scotia populations.

	Female			
	Manitoba		Nova Scotia	
Individual (mm)	Body Length	Wingspan	Body Length	Wingspan
1	15.11	44.05	22.21	45.08
2	22.37	45.23	20.08	47.69
3	19.30	44.81	23.56	47.24
4	23.25	53.44	26.30	45.59
5	18.02	41.12	23.38	50.49
Mean	19.61	45.73	23.11	47.22

The male adults of *A. virginiensis* are typically smaller than the females. Below, Table 3 shows the measurements of the wingspans and body lengths from the males of the Manitoba and Nova Scotia populations. By looking at the table one can see that the male mean body length and wingspan is slightly higher in the Nova Scotia population.

Table 3. Wingspans and body lengths (in mm) of adult male *A. virginiensis* from the Manitoba and Nova Scotia populations.

	Male			
	Manitoba		Nova Scotia	
Individual (mm)	Body Length	Wingspan	Body Length	Wingspan
1	18.19	29.75	19.35	33.43
2	19.69	30.76	17.82	30.50
3	20.07	32.78	18.21	32.01
4	17.44	28.07	17.56	31.41
5	18.47	30.77	18.01	32.75
6	17.89	32.89		
Mean	18.63	30.84	18.19	32.02

Table 4. Wingspans and body lengths (in mm) of adult female *Anisota* from the United States.

Female			
Location	Body Length	Wingspan	Species
Michigan, USA	21.65	55.03	*Anisota virginiensis*
Michigan, USA	21.02	48.9	*Anisota virginiensis*
Michigan, USA	21.95	53.83	*Anisota virginiensis*
South Carolina, USA	22.25	47.08	*Anisota virginiensis*
Louisiana, USA	24.13	45.24	*Anisota virginiensis*
North Carolina, USA	23.18	47.39	*Anisota virginiensis*
North Carolina, USA	19	47.47	*Anisota virginiensis*
Mean	21.88	49.28	

Table 5. Wingspans and body lengths (in mm) of adult male *Anisota* from the United States.

Male			
Location	Body Length	Wingspan	Species
Florida, USA	16.01	37.78	*Anisota v. pellucida*
South Carolina, USA	16.81	35.97	*Anisota virginiensis*
Gainesville, USA	18.22	31.31	*Anisota pellucida*
Mean	17.01	35.02	

Using Microsoft Excel, a two-sample t-test assuming unequal variances were carried out to compare the male body lengths, male wingspans, female body lengths, and female wingspans. A two-tailed alpha value of 0.025 was used for every test. Males from both *A. virginiensis* populations were tested against one another as well as females. The USA male and female populations were also tested against both the Manitoba and Nova Scotia populations.

Table 6 displays the two tailed, unequal variances t-test conducted for male body lengths (Manitoba vs. Nova Scotia) resulted in a p-value of 0.43 (no significant difference).

Table 6. Two-tailed t-test table of male body lengths.

	Variable 1	*Variable 2*
Mean	18.625	18.19
Variance	1.07599	0.47805
Observations	6	5
Hypothesized Mean Difference	0	
df	9	
t Stat	0.82960045	
P(T<=t) one-tail	0.21410982	
t Critical one-tail	1.83311293	
P(T<=t) two-tail	0.42821964	
t Critical two-tail	2.26215716	

Table 7 shows the two-tailed, unequal variances t-test conducted for male wingspans (Manitoba vs. Nova Scotia) resulted in a p-value of 0.23 (no significant difference).

Table 7. Two-tailed t-test table of male wingspans.

	Variable 1	*Variable 2*
Mean	30.8366667	32.02
Variance	3.36766667	1.3009
Observations	6	5
Hypothesized Mean Difference	0	
df	8	
t Stat	-1.305613	
P(T<=t) one-tail	0.11398865	
t Critical one-tail	1.85954804	
P(T<=t) two-tail	0.22797731	
t Critical two-tail	2.30600414	

Table 8 displays the two tailed, unequal variances T-test conducted for female body length (Manitoba vs. Nova Scotia) resulted in a P-value of 0.09 (no significant difference).

Table 8. T-test table of female body length.

	Variable 1	*Variable 2*
Mean	19.61	23.106
Variance	10.93535	5.11058
Observations	5	5
Hypothesized Mean Difference	0	
df	7	
t Stat	-1.9515244	
P(T<=t) one-tail	0.04598041	
t Critical one-tail	1.89457861	
P(T<=t) two-tail	0.09196083	
t Critical two-tail	2.36462425	

Table 9 shows the two-tailed, unequal variances t-test conducted for female wingspan (Manitoba vs. Nova Scotia) resulted in a p-value of 0.54 (no significant difference).

Table 9. T-test table of female wingspans.

	Variable 1	*Variable 2*
Mean	45.73	47.218
Variance	21.15375	4.53767
Observations	5	5
Hypothesized Mean Difference	0	
df	6	
t Stat	-0.6564382	
P(T<=t) one-tail	0.26794552	
t Critical one-tail	1.94318028	
P(T<=t) two-tail	0.53589103	
t Critical two-tail	2.44691185	

Table 10 shows the two-tailed, unequal variances t-test conducted for female body lengths in the Manitoban and USA populations resulted in a p-value of 0.22 (no significant difference).

Table 10. T-test table of Manitoban and USA female body lengths.

	Variable 1	*Variable 2*
Mean	19.61	21.8828571
Variance	10.93535	2.66352381
Observations	5	7
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.4184391	
P(T<=t) one-tail	0.10763554	
t Critical one-tail	2.01504837	
P(T<=t) two-tail	0.21527108	
t Critical two-tail	2.57058184	

Table 11 shows the two-tailed, unequal variances t-test conducted for female wingspans for the Manitoban and USA populations resulted in a p-value of 0.20 (no significant difference).

Table 11. T-test of Manitoban and USA female wingspans.

	Variable 1	*Variable 2*
Mean	45.73	49.2771429
Variance	21.15375	13.6531905
Observations	5	7
Hypothesized Mean Difference	0	
df	7	
t Stat	-1.4267309	
P(T<=t) one-tail	0.09835032	
t Critical one-tail	1.89457861	
P(T<=t) two-tail	0.19670063	
t Critical two-tail	2.36462425	

Table 12 shows the two-tailed, unequal variances t-test conducted for male body lengths for the Manitoban and USA populations resulted in a p-value of 0.11 (no significant difference).

Table 12. T-test of Manitoban and USA male body lengths.

	Variable 1	*Variable 2*
Mean	18.625	17.0133333
Variance	1.07599	1.25203333
Observations	6	3
Hypothesized Mean Difference	0	
df	4	
t Stat	2.08643999	
P(T<=t) one-tail	0.05262672	
t Critical one-tail	2.13184679	
P(T<=t) two-tail	0.10525344	
t Critical two-tail	2.77644511	

Table 13 shows the two-tailed, unequal variances t-test conducted for male wingspans for the Manitoba and USA populations resulted in a p-value of 0.14 (no significant difference).

Table 13. T-test of Manitoban and USA male wingspans.

	Variable 1	*Variable 2*
Mean	30.8366667	35.02
Variance	3.36766667	11.1421
Observations	6	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-2.023199	
P(T<=t) one-tail	0.06811739	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.13623479	
t Critical two-tail	3.18244631	

Table 14 shows the two-tailed, unequal variances t-test conducted for female body lengths for the Nova Scotia and USA populations resulted in a p-value of 0.34 (no significant difference).

Table 14. T-test of Nova Scotian and USA female body lengths.

	Variable 1	*Variable 2*
Mean	23.106	21.8828571
Variance	5.11058	2.66352381
Observations	5	7
Hypothesized Mean Difference	0	
df	7	
t Stat	1.03277868	
P(T<=t) one-tail	0.16803189	
t Critical one-tail	1.89457861	
P(T<=t) two-tail	0.33606378	
t Critical two-tail	2.36462425	

Table 15 shows the two-tailed, unequal variances t-test conducted for female wingspans for the Nova Scotia and USA populations resulted in a p-value of 0.25 (no significant difference).

Table 15. T-test of Nova Scotian and USA female wingspans.

	Variable 1	*Variable 2*
Mean	47.218	49.2771429
Variance	4.53767	13.6531905
Observations	5	7
Hypothesized Mean Difference	0	
df	10	
t Stat	-1.2180248	
P(T<=t) one-tail	0.12558628	
t Critical one-tail	1.81246112	
P(T<=t) two-tail	0.25117256	
t Critical two-tail	2.22813885	

Table 16 shows the two-tailed, unequal variances t-test conducted for male body lengths for the Nova Scotia and USA populations resulted in a p-value of 0.20 (no significant difference).

Table 16. T-test of Nova Scotian and USA male body lengths.

	Variable 1	*Variable 2*
Mean	18.19	17.0133333
Variance	0.47805	1.25203333
Observations	5	3
Hypothesized Mean Difference	0	
df	3	
t Stat	1.64291106	
P(T<=t) one-tail	0.09947223	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.19894445	
t Critical two-tail	3.18244631	

Table 17 shows the two-tailed, unequal variances t-test conducted for male wingspans for the Nova Scotia and USA populations resulted in a p-value of 0.27 (no significant difference).

Table 17. T-test of Nova Scotian and USA male wingspans.

	Variable 1	*Variable 2*
Mean	32.02	35.02
Variance	1.3009	11.1421
Observations	5	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.5048585	
P(T<=t) one-tail	0.13564346	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.27128693	
t Critical two-tail	4.30265273	

Wing patterns and colouration are typically bolder in the adult male moth. The wing colouration within the males and females of both populations are darker in the Nova Scotia population. Male moths have a clear transparent patch in their forewings with a white dot in the middle and purple tips. The purple is much deeper in the Nova Scotia population. The orange body colouration is very similar between both male populations. The forewings between male moth populations also show a difference in the shape. In the Manitoba males the forewings are convexly shaped along the edges, whereas the Nova Scotia males the forewing edges are relatively straight, shown in Figure 8. Figure 8 shows a comparison between the two male moth populations.

Figure 8. Forewing edge comparison between Manitoba male (left), Nova Scotia male (right). Source: Claire Hensrud.

Female moths do not have a transparent patch in their wing as they are nocturnal. Similar to the males, female moths have a white dot in the middle of their forewing. Female wings are split into two colours, light purple on the outside edge and orange in the middle divided by a darker purple line. By comparing the Manitoba and Nova Scotia populations it is clear that the Nova Scotia population has a more pigmented wing colour. The antemedial lines in the Nova Scotia females are far narrower than the ones in the Manitoba population, shown in Figure 9. The bodies of both populations are a very similar light orange colour, lighter than the male bodies. Figure 9 shows a comparison between the Manitoba and Nova Scotia females.

Figure 9. Antemedial line comparison between Manitoba female (left), Nova Scotia female (right). Source: Claire Hensrud.

The caterpillars of *A. virginiensis* (Figure 10) also show a slight variation in colour. Similar to the moths, the Nova Scotia caterpillars appear to have a darker pigmentation within their body stripes. The Nova Scotia caterpillar has darker black stripes as well as more pink in its coloured stripes.

Figure 10. *Anisota virginiensis* caterpillars from Nova Scotia (left) and Manitoba (right). Source: Don Henne.

There are fairly distinct morphological differences between the males and females of the Manitoba and Nova Scotia specimens in comparison to the USA specimens. It is clear the USA specimens are typically darker in colour than the males and females of both Canadian populations. Within the male comparisons, again the Manitoba moths have a convex shaped outer wing edge, and the Nova Scotia moths have a straight outer wing edge. The USA moths have even straighter outer wing edges on both the forewings and hindwings (Figures 11 and 12). Although the Nova Scotia moths are a dark purple colour, the USA moths have an even darker purple colouration.

Figure 11. Manitoba male moth (left) and USA male moth (right). Source: Claire Hensrud.

Figure 12. Nova Scotia male moth (left) and USA male moth (right). Source Claire Hensrud.

The colouration of the USA female moths are darker than those of the Manitoba female moths (Figure 13) but fairly comparable to the Nova Scotia moths (Figure 14). The USA moths also appear to be longer than those of the Canadian female moths. The antemedial lines are narrower than the Manitoban moths but slightly larger or very comparable to the Nova Scotia moths.

Figure 13. Manitoba female moth (left) and USA female moth (right). Source: Claire Hensrud.

Figure 14. Nova Scotia female moth (left) and USA female moth (right). Source: Claire Hensrud.

DISCUSSION

Anisota virginiensis proved to exhibit a copious amount of sexual dimorphism as it was stated in Ferguson's (1971) literature. In a study done by Costa, et al. (2008), the shape of clams from different populations were compared. The overall shell shape differences of individuals were examined from different populations. *Ruditapes decussatus* and *Ruditapes philippinarum* were compared. Using the left value from each individual profile was created from photographs. It was found that different environmental factors can affect the morphology of bivalve shells such as, depth, water turbulence, wave exposure and sediment type to name a few (Costa et al. 2008).

Within the order Lepidoptera, wing colour is affected by a number of factors such as, defense, mating, and mimicry (Ellers and Boggs 2002). Typically, the intraspecific variation of wing colouration is an adaptation to the local environmental conditions. It is possible for local selection pressures to cause consequences such as reproductive isolation. It was found by Ellers and Boggs (2002) that wing coloration strongly diverged at higher elevations. The amount colour-pattern diversity of a population of *Zizeeria maha* increased in certain areas due to temperature stress (Hiyama, et al. 2012).

In my study it was also found that not only is there a difference between males and females, but there is a difference between the males and females of the two separate Canadian populations. The colouration and wing morphology of the adult moths are clearly distinct between the Nova Scotia and the Manitoba populations. Both the males and females of the Nova Scotia population are darker in colour, making their wings more distinct. The Manitoba population has more muted colours. The wing patterns were the same in both the male and female moths. Males have the transparent patch on their forewing, purple edges and a white dot in the middle. Females have purple edges and an orange middle with a dark purple line separating the colours and a white dot in the middle of their forewing. There is a significant morphological difference between the forewings of the male moths. The Manitoba specimen had a more convex shaped wing, whereas the Nova Scotia specimen had relatively straight edges. The antemedial lines in the Nova Scotia females are far narrower than the ones in the Manitoba population. Variation in wing colour and patterns is strongly affected by the local environmental conditions (Ellers and Boggs 2002), which is likely what is occurring amongst these populations. It is possible that the adult *A. virginiensis* females overall have slightly less pigmentation on their wings than the males do because of the time they are active as females are nocturnal and the males are diurnal (Ferguson 1971).

It is noted that even with no significant difference between tested populations due to variance within local populations, that adult male and female moths from the Nova Scotia population had generally larger body lengths and wing spans than those from the Manitoba population. Looking at the moths (Figures 9 and 10), one can see differences in size yet there was no significant difference found in the t-tests. This implies that the sample size was likely not large enough. It could be possible that local morphological variations could be better accounted for with larger sample sizes from each area. As discussed in Costa el al. (2008) different environmental factors can affect the morphology of a species. Seeing as Manitoba and Nova Scotia are located in very different parts of Canada, it is likely environmental factors such as temperature and precipitation, as well as latitude play a role in the differentiation between populations.

In t-tests performed on the Canadian vs. USA data, there were still no significant differences between wingspans and body length. It is apparent there is a morphological difference amongst the different populations (Figures 12-15), which is also likely a reflection on the environmental factors each population faces. The USA specimen are darker in colour in both the males and females. The outer wing edges of the males are very straight in both the forewings and hindwings (Figures 12 and 13).

This study originally planned on performing a mitochondrial DNA analysis on the *A. virginiensis* moths of both sexes from both the Manitoba and Nova Scotia populations. This would help to provide further findings on whether or not there is a divergence occurring within the species between the Manitoba and Nova Scotia populations. Unfortunately, this was unable to be put in motion due to COVID-19 and it causing our inability to find a collaborator to perform the DNA analysis.

CONCLUSION

While no statistically significant differences were found in this experiment, there is an option to do further research. This project is just the beginning as to what could be done in this area of research. With a larger sample size, results could show a clearer direction as to whether or not these two populations are a result of one species diverging into two (or more). Performing mitochondrial DNA analysis is another way one could determine how closely related these two populations are. DNA is coded and made into species specific DNA barcodes allows a comparison between codes to determine if there is a difference. Overall, there was no significant difference found in either the wingspan or body size of both the males and females in the Manitoba or Nova Scotia populations, but there are opportunities to conduct further interesting research by collecting more data from more populations throughout the range of *A. virginiensis*. Future research should also consider doing qualitative assessments of the morphological traits to test not only size, but the shape and colour patterns of the moths to help distinguish distinctive trends between the populations.

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