THERMAL ADAPTATION IN THE AMERICAN RED SQUIRREL (*TAMIASCIURUS HUDSONICUS*)

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THERMAL ADAPTATION IN THE AMERICAN RED SQUIRREL

(*TAMIASCIURUS HUDSONICUS*)

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Temperature is one of the most important environmental variables impacting organisms. For

endotherms, temperature can be extremely testing since they need to maintain a constant core

body temperature at any given ambient temperature. However, endotherms can survive both in

very hot areas and very cold areas and mammals are, for example, found throughout the world

and widespread species may face temperature differences of more than 80C over their

distribution range. Recent changes in climate are likely to have great impact on thermoregulatory

abilities and with increased regional temperatures due to anthropogenic climate change more and

more species are being pushed to, or beyond their thermal limits in parts of their distribution,

making an understanding of adaptation to temperature particularly important.

The following series of studies examine multiple aspects of thermal adaptation in the

American red squirrel (*Tamiasciurus hudsonicus*). The first chapter asks if the distribution of the

species is limited by temperature. Indeed, three of the major predictors of the species distribution

reflect temperature, i.e. mean annual temperature, minimum temperature of the coldest month

and maximum temperature of the warmest month. Knowing the importance of climate in

determining the species niche helps us understand the selective pressures it asserts. The second

and third chapters discuss adaptations found in the morphology of *T.hudsonicus* that relate to

temperature. Both whole body and skull dimensions vary with temperature, with smaller animals

being found in colder areas, either as a response to reduction of heat loss in cold conditions or

increased heat loss in hot conditions. This is consistent with Bergmann's Rule, but with regards Allen's Rule, only one protuberance or appendage – the nose - was found to show an adaptive trend and that shows a relationship with humidity rather than temperature. The fourth chapter examines variation in gene expression levels in response to temperature and elevation. The gene expression responses suggest that *T.hudsonicus* has a decreased metabolic activity in cold conditions compared to warm (or alternatively increased metabolic activity in warm conditions) and shows greater metabolic responses to increased elevation than to temperature.

Overall *T.hudsonicus* is found to live within limits determined by temperature and shows both morphological and gene expression response to temperature.

BIOGRAPHICAL SKETCH

Fríða Jóhannesdóttir was born in Iceland late July during Iceland's one week of summer. She lived close to the Arctic Circle in Akureyri until moving to the capital city, Reykjavík, to study Biology at the University of Iceland. This decision was made based on her interest in genetics following the cloning of Dolly the sheep and the founding of DeCode. Thankfully Professor Páll Hersteinsson showed her the light and turned her in the direction of mammal ecology prompting her to move to Scotland to finish her BSc degree at the University of Aberdeen. Following her time in Scotland Fríða took a year off from science and worked with children with special needs before deciding that the University system might have more need for her skill than the pre-schools. She therefore moved again to the UK to complete a Masters in Ecology and Environmental Management at the University of York. It was during that time she learnt that not all genetic studies have to involve *Drosophila* but can focus on crucial issues such as looking at the evolutionary ecology of small mammals. With that in mind she joined the lab of Jeremy Searle which led her to Cornell to do a PhD with him there. At Cornell Fríða was determined to understand how a widespread mammal can deal with a wide temperature range, a question close to her heart due to her inability to thermoregulate. After a false start on a European species she sat down with a mammal guide looking for a species that would have appropriate distribution and ecology to answer the question and chose the cutest one, the American red squirrel. Fríða thesis work took her to the corners of the species distribution, allowing her to visit 36 states in the US and 8 provinces of Canada during her stay in North America.

This thesis is dedicated to Yorkshire tea.

Without it, all of this would never have happened.

May it continue supporting me through science for a long time to come.

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I would have been unable to complete this work without help from a large number of people both at museums and field location. I am first of all grateful to Charles Dardia (Cornell University Museum of Vertebrates) who has been immensely supportive throughout my time in Ithaca helping with specimen preparation, and helping me and my field assistants get certification for collecting. Charles was also supportive during my time as a lab instructor for the Mammalogy course. Additionally, Eileen Westwig (American Museum of Natural History), William Stanley and Rebecca Banasiak (Field Museum of Natural History) and Suzanne Peurach (National Museum of Natural History) provided invaluable assistance while obtaining museum

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CHAPTER 1

The thermal niche of the American red squirrel (*Tamasciurus hudsonicus*)

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ABSTRACT

Temperature is one of the most important environmental variables impacting organisms. For endotherms, temperature can be extremely testing since they need to maintain a constant core body temperature at any given ambient temperature. Mammals are found throughout the world and widespread species may face temperature differences of more than 80C over their distribution range. To cope with these extreme temperatures mammals may either respond behaviorally or physiologically. If these responses prove unsuccessful in maintaining a constant body temperature the individual will perish.

In this study we focused on the American red squirrel, *Tamiasciurus hudsonicus*, a small widespread mammal found in coniferous forests of North America. The species is active year round and found as far south as southern New Mexico and Georgia (in mountain ranges) and as far north as northern Alaska. Here we attempt to answer how temperatures affects the species distribution range using species distribution models.

Our findings suggest that climate is the main predictor of the distribution of *T.hudsonicus*. We found that three main climatic factors; annual mean temperature, maximum

temperature of the warmest month and the minimum temperature of the coldest month were the most important factors in our species distribution model. While the species can easily cope with cold temperatures it appears that it may sometimes be close to its heat limits and it can be expected that with increased temperature rise, due to anthropogenic climate change, populations in the south, which already are isolated on mountain tops, may go extinct.

INTRODUCTION

One of the most important environmental variables to affect organisms in general, and mammals in particular, is temperature, which can change daily, seasonally or spatially. In polar areas the environmental temperature can be as low as 60-70C below zero and in hot deserts temperature at the hottest time of the year can reach between 50 and 60C (Careau 2007). These extremes mean that mammals are subjected to great physiological challenges and temperature can influence individual behavior and fitness and, when individuals are pushed beyond their thermal limits, may cause cold and heat stress (Karl & Fischer 2008; Marcos-Carcavilla et al 2010). The thermal limits are between the lower critical temperature, i.e. the lowest temperature a mammal can withstand without increasing metabolic rate to maintain constant body temperature, and the upper critical temperature, i.e. the highest temperature a mammal can withstand before starting heat dissipation (Feldhamer et al 2016). In essence, the temperature limits in which a species can survive defines its 'thermal niche' and this concept can be applied to mammals as to other taxa (Bowler et al 2015).

Mammals are endotherms and as such use metabolic heat to regulate body temperature, keeping it constant irrespective of the environmental temperature (McNab 2002). This provides the benefit of high aerobic capacity and extended periods of activity but it is energetically expensive and the main challenges when dealing with temperature extremes are related to

management of energy and water flow (Humphries et al 2005; Careau 2007). When faced with temperatures lower than the critical temperature and the endotherms are losing heat to the environment they need to increase their energy expenditure and heat production and thus metabolism (e.g. Humphries et al 2005; Careau 2007; Moore & Huntington 2008). Some mammals deal with decreased temperature by lowering body temperature and increasing both insulation and fur thickness (Lovegrove 2005; Glanville & Seebacher 2010). However, species may instead avoid exposure to extreme cold and thereby decrease energy demands for thermoregulation and need for increased foraging. In hot areas, where water scarcity is often a concurrent problem, ambient temperature is often close to the core body temperature. One of the most efficient ways of dealing with excess metabolic heat is through evaporation (since convection becomes problematic when the thermal gradient is reduced) which, in dry areas, can increase risk of dehydration (Careau 2007).

Even though endotherms like mammals have multiple ways of dealing with various thermal conditions there are limits to what each species can tolerate and this constrains species distributions (Humphries, Thomas & Speakman 2002; Gammons, Mengak & Conner 2009). Species need to spend more energy to maintain body function beyond their thermoneutral zone (Feldhamer et al 2016), they can only do so for short periods of time without a significant loss of fitness and will not be able to survive long-term in areas outside of their thermoneutral zone (Oswald et al 2010). Species with narrow distribution are often found to have lower thermal tolerance than widespread species that may face extreme cold during the winter in parts of the species distribution and extreme heat in summer in others (Sunday, Bates & Dulvy 2012). However it has also been reported that there may not be any relationship between the breadth of the thermoneutral zone and latitude, and climatic variability, meaning that thermal physiology of

mammals may not be particularly linked to ambient temperature and the species may instead have behavioral strategies to cope with temperature (Kjaliq et al 2014).

Given those contrasting results it is of great interest to analyze the importance of temperature and other climatic variable on particular species of mammal. A way of understanding the relationship between ecological factors, climate, and the distribution of particular species is to use ecological niche models/species distribution models (Loveless et al 2016). These use current location data for a species and use geographic information systems (GIS) to attribute environmental data (topography, climate, land cover category etc.) to the location data and from that build a model that accurately describes the species distribution. Species distribution models have been applied widely for mammals (e.g. Kanagaraj et al 2013; Latinne et al 2015). In relation to an interest in defining the thermal niche, species distribution models can, in particular, provide information on the impact of temperature in constraining the species distribution.

A species with an exceptionally wide distribution, and therefore of great interest in terms of its response to climate, is the America red squirrel (*Tamiasciurus hudsonicus*). This is a small mammal found in coniferous forests throughout northern North America and along the Appalachian mountain chain in the east down to Georgia and the Rocky Mountains in the west down to New Mexico (Steele 1998). The species is found as far north as Aklavik, Northwest Territories (around 68N), as far west as the Seward peninsula, Alaska (up to 168W), as far south as the Mogollon mountains, New Mexico (around 33N), and as far east as Newfoundland (around 52W). The species does not hibernate or go into daily torpor and needs to leave the nest to forage for food throughout the year. For these reasons the species is subjected to both extreme cold temperatures in various parts of its distribution, and to high temperatures in others. It can

therefore be suggested that the species limits might be determined, in part, by temperatures, potentially cold temperature in the north and warm temperatures in the south. Here we examine this contention using a species distribution model based on location data from museum records of *T.hudsonicus*.

MATERIALS AND METHODS

Species records

Presence data for *T.hudsonicus* over the period 1960-2000 was obtained from a variety of museums, both through vertnet.org and from museum curators and collection managers and represent a subset of specimens in a larger study with museum records over ca. 150 years (chapter 2). This 40-year period was chosen to fit with the accurate climate information available. The 2681 *T.hudsonicus* records that have been gathered cover the known range for the species throughout USA and Canada (figure 1.1). There is an issue of spatial auto-correlation (SAC) with such data. SAC can occur when certain areas are more intensively surveyed leading to a clustering of observations (Merow, Smith & Silander 2013). SAC causes model biases by artificially inflating the ecological conditions of the correlated observation (Elith, Kearney & Phillips 2010). To eliminate SAC from calculations we employed the SDM toolbox package for ArcGIS 10.1 (ESRI 2012) where an analysis using buffers was conducted in the context of the spatial heterogeneity of the environment, deleting records with duplicate environmental information but retaining those with unique information (Brown 2014).
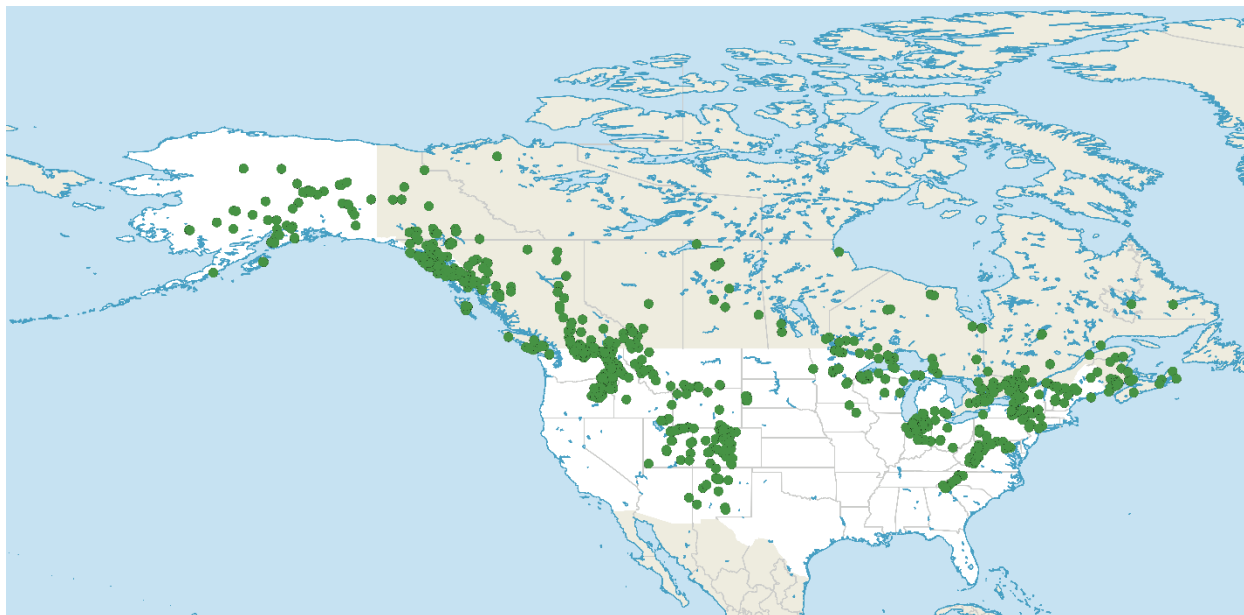
Figure 1.1 – Locations of specimens used in the study.

Environmental data

We chose a set of environmental predictors associated to the occurrence of *T.hudsonicus*. We incorporated all 19 climatic variables from the WorldClim database (http://www.worldclim.org), the digital elevation data of the NASA Shuttle Radar Topography Mission (http://srtm.csi.cgiar.org) as a topographical variable and 10 land cover variables from the Globcover project (http://postel.obs-mip.fr/?GLOBCOVER-Project) including a mix of land cover types where *T.hudsonicus* is likely to occur, may or may not occur and is unlikely to occur, based on the field biology of the species (Reid 2006). All variables had a resolution of 30 arc-seconds (ca. 1 km) and all GIS processing was made in ArcGIS 10.1 (ESRI 2012).

It was an essential step of this study to avoid multicolinearity among the environmental predictors, for which we calculated a correlation matrix. Using the Pearson correlation (*r*) value of 0.8 as a threshold (Dormann et al 2013), only one out of a group of correlated variables was kept as a predictor, and it is the variable with the greatest impact (based on a principal component analysis) that was retained. All calculations were made in the SDM toolbox for

ArcGIS 10.1 (ESRI 2012). From the collinearity analyses we kept 10 environmental predictors (table 1.1).

To test the effect of different environmental predictors we used three datasets of predictors for calculating the model: one only containing climatic variables (climatic model), another with land cover and elevation (landscape model) and a last with all environmental predictors (full model).

Table 1.1 – Uncorrelated environmental predictors used for the species distribution modelling analyses

Type	Description	Short name
	Annual Mean Temperature	Bio1
	Max Temperature of Warmest Month	Bio5
	Min Temperature of Coldest Month	Bio6
	Temperature Annual Range	Bio7
Climatic	Mean Temperature of Warmest Quarter	Bio10
	Annual Precipitation	Bio12
	Precipitation of Driest Quarter	Bio17
	Precipitation of Warmest Quarter	Bio18
Topography	Elevation	Elev
Habitat	10 land cover classes*	Land

*Land cover classes: Broadleaf forest, Coniferous forest, Shrubland, Coniferous shrubland, Grassland, Cropland, Urban water bodies, Snow and ice, Wetlands

Species distribution modelling

Species distribution models were built using the maximum entropy modelling technique, Maxent version 3.3.3k (Phillips, Anderson & Schapire 2006), which outperforms other modelling methods (Hernandez et al 2006; Wisz et al 2008; Rebelo & Jones 2010). Species presence records and the selected environmental predictors (table 1.1) were imported into Maxent and run in auto features with a regularization multiplier of 1. Then, 10 model replicates were run using cross-validation. The area under the curve (AUC) of the receiver operating characteristics (ROCs) plot was taken as a measure of the overall fit of the models (Fielding & Bell 1997; Phillips, Anderson & Schapire 2006). The 10 variables already selected (table 1.1) were then

used to build the final models. The cross-validation approach is an alternative way of testing

models as the use of 'training' and 'testing' datasets (e.g. Elith, Kearney & Phillips 2010).

Importance of variables

The importance of each variable was measured using the percent contribution to the model and

the jackknife values of the regularized training gain (a measure of likelihood between species

data and the variable).Variable response curves were determined from univariate models created

by Maxent.

RESULTS

The climatic and full models performed well (climatic: AUC=0.867±0.022, full:

AUC=0.877±0.012) while the landscape model achieved a poorer performance

(AUC=0.722±0.02). Both climatic and full models predicted the occurrence of *T.hudsonicus*

over the main mountain ranges of North America (figures 1.2 – 1.3). Because of the poorer

performance of the landscape model, we do not show a map based on that.

The environmental predictors that contributed most highly to explain the occurrence of

T.hudsonicus were three climatic variables associated to temperatures (Bio1: annual mean

temperature, Bio5: maximum temperature of the warmest month, Bio6: minimum temperature of

the coldest month). The importance of these predictors is evident from both the percent

contribution values and the jackknife values of the regularized training gain in the full model

(figure 1.4). The three most important temperature predictors each showed a unimodal response

underlying the occurrence of squirrels (figure 1.5). Interestingly the topographic predictor

(elevation) shows almost no contribution to explain the occurrence of *T.hudsonicus* (figure 1.4).

Land cover also has a much smaller predictive value than the top temperature predictors, with the

presence of forests (conifer and broadleaved) and proximity to water bodies the most important

variables (data not shown).
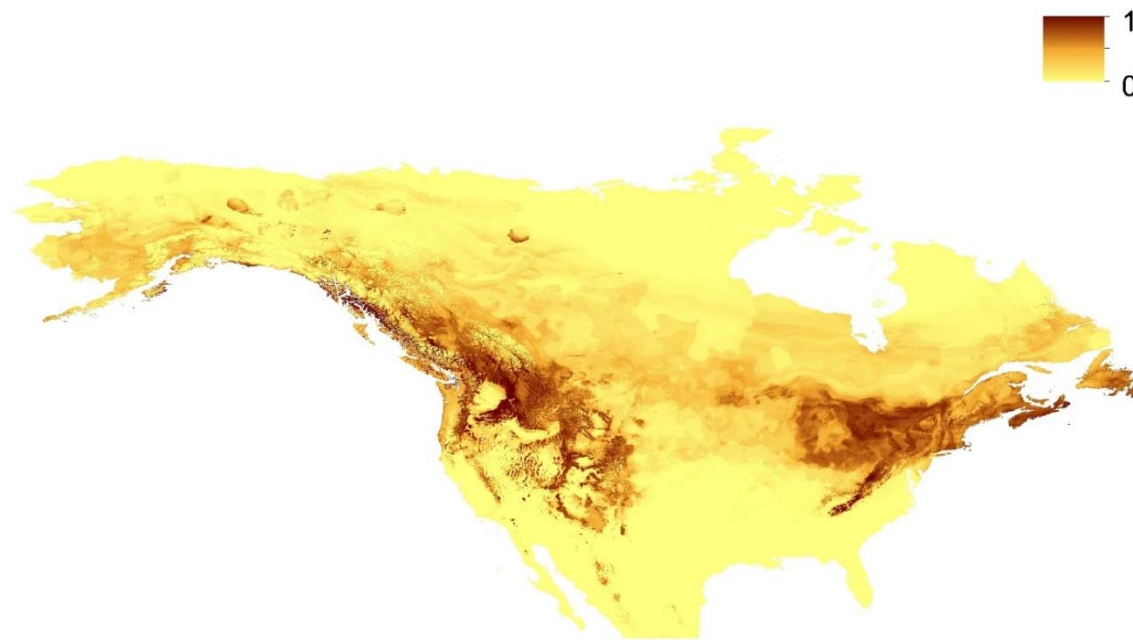
Figure 1.2 – Climatic species distribution model of T.hudsonicus *for North America. Darker colors indicate greater probability of occurrence, ranging from 0 where the species is predicted absolutely to be absent to 1 where the species is predicted absolutely to be present.*
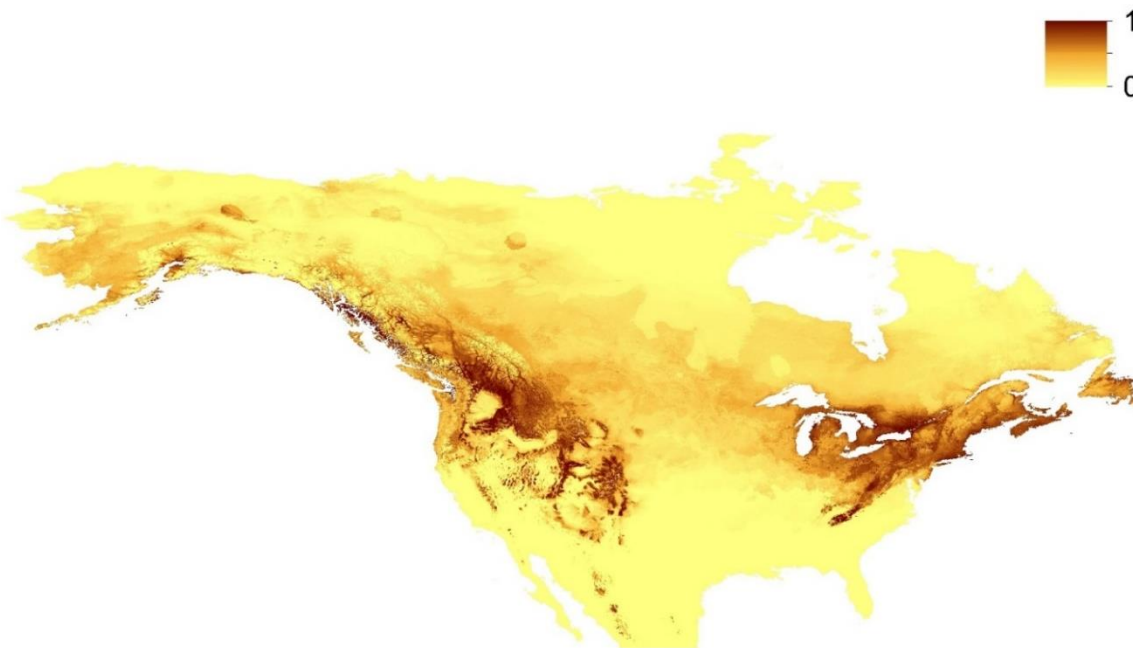
Figure 1.3 – Full species distribution model of T.hudsonicus *for North America. Darker colors indicate greater probability of occurrence, ranging from 0 where the species is predicted absolutely to be absent to 1 where the species is predicted absolutely to be present.*
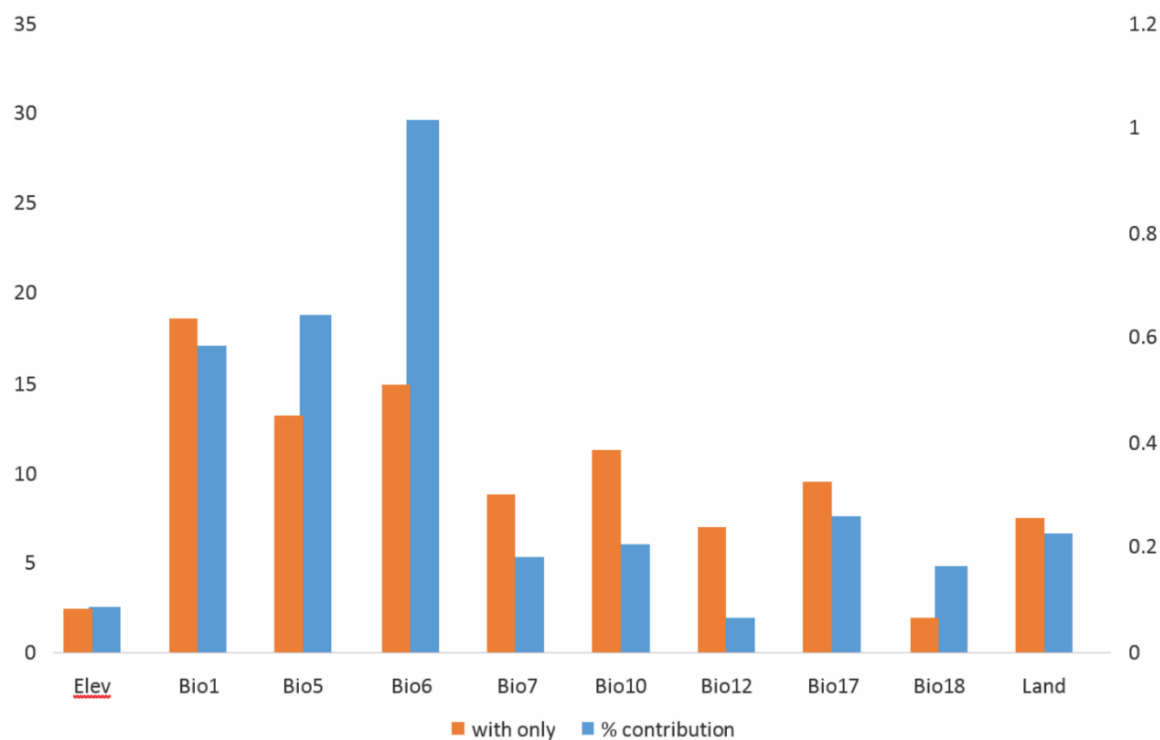
Figure 1.4 – Importance of each variable (short name according to table 1.1) for the full species distribution model. Percentage contribution of each variable (% contribution; left axis) and values of the jackknife results for models for only the one variable (with only; right axis).
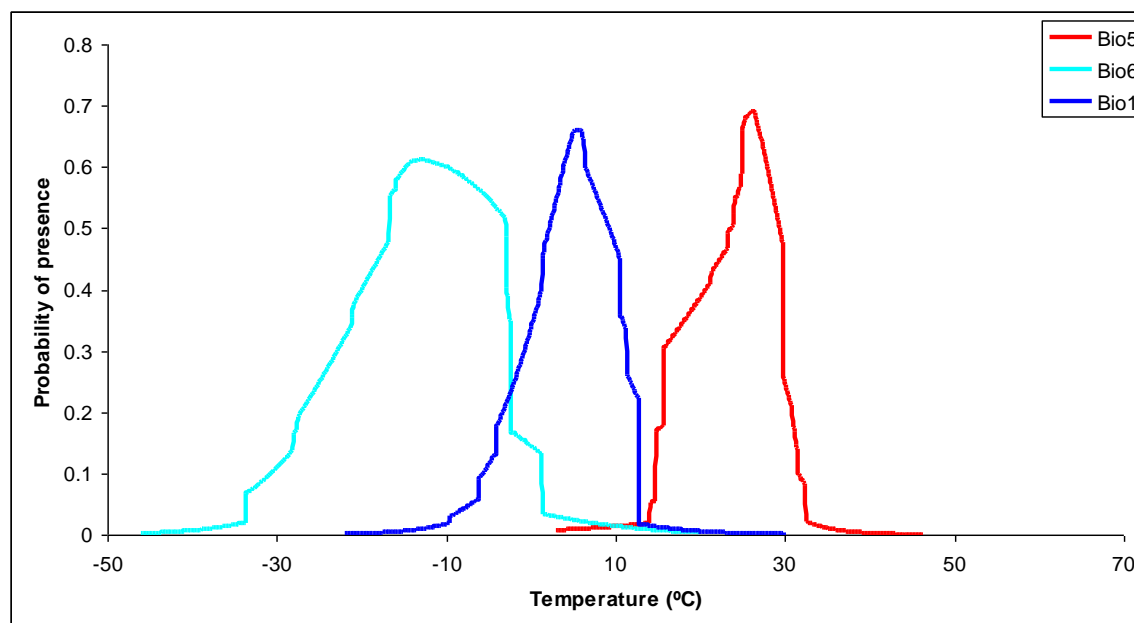
Figure 1.5 – Response curves of the best supported climatic predictors. Bio1 is the annual mean temperature, Bio5 is the maximum temperature of the warmest month and Bio6 is the minimum temperature of the coldest month.

DISCUSSION

Of the three models created here, the full and climatic species distribution models performed well while the landscape model performed badly. This has been found in other studies (Pearson & Dawson 2003): at a continental scale climate related factors are more likely to be important than land cover, which is more relevant at a local domain. *T.hudsonicus* occurs over much of North America and so can be considered to have a distribution that is continental in scale. Topography is relevant at intermediate geographical scales (Pearson & Dawson 2003) and it is interesting how unimportant elevation, as a topographical factor, is to the full distribution model. Although the distribution of *T.hudsonicus* clearly relates to elevation with occurrence in the Rocky Mountains and Appalachians (figures 1.2 and 1.3), it is actually temperature which appears to be determinant of this distribution.

In terms of the individual environmental predictors used in the species distribution model, annual mean temperature (Bio1), maximum temperature of the warmest month (Bio5), and minimum temperature of the coldest month (Bio6) are the most important. These are the primary factors defining the 'species temperature niche' or 'thermal niche' of *T.hudsonicus* (see Bowler et al 2015). In a North American context, it is clear from the response curves (figure 1.5) that *T.hudsonicus* is a species that can tolerate very low winter temperatures, but is not found where there are high summer temperatures. In fact Bio5 predicts that there is a sharp decline in species occurrence at 30C, with the species not being found in areas where the average hottest summer temperature reaches beyond 32C, while Bio6 predicts a gradual decline in occurrence from around -12C to approximately -33C below which the species will not be found. This fits extremely well with the species distribution. The temperature range described for *T.hudsonicus* goes from an average coldest temperature during January below or around -30C in the far north in the

distribution to around 30C as the average highest temperature in July in the southern part of the species distribution (http:www.usclimatedata.com). The species is not found in areas colder than those or warmer than those (although the species will survive short periods above or below these thresholds). *T.hudsonicus* is distributed throughout northern North America but is limited to high ground (Rocky Mountains, Appalachians) further south, reflecting that.

Hope et al (2016) independently constructed a species distribution model for *T.hudsonicus*. However it was based on a smaller number of individuals from a wider timescale than ours and was used for different purposes (particularly in relation to a hypothesized subdivision of *T.hudsonicus* and incorporation of other *Tamiasciurus*, and paleodistributions). There is not the same focus on the thermal niche.

Individuals in a warm climate are already close to the limits of their thermal tolerance and the distribution of *T.hudsonicus* is extremely influenced by the highest summer temperature. We can see that in figure 1.5, with the sharp decline in the response curve at about 30C. Sherwood and Huber in 2010 estimated that even mammals as environmentally labile as humans will in the future face intolerable conditions due to heat stress. Net conductive and evaporative cooling can only occur if an animal is warmer than the minimum temperature reachable with evaporative cooling (T_w) meaning if an animal finds itself in an environment where T_w exceeds its own temperature it cannot lose heat to the environment. For heat to be dissipated to the environment the temperature of the skin must be colder than the core body temperature. In the case of humans the skin temperature never exceeds 35C under normal conditions. Environmental temperatures above regulated skin temperature for any extended time will lead to elevated core temperature and hyperthermia. This is relevant to anthropogenic climate change. With predicted increase in temperature there are several areas where *T.hudsonicus* is now found where it will

most likely face increased risk of heat stress and will not be able to survive in those places. The species may be able to cope physiologically with the changed environmental conditions by phenotypic plasticity or genetic change (Visser 2008), but there are limits to the extent to which a species can adapt to changed thermal conditions and therefore local extinctions are inevitable (Parmesan 2006; Sherwood & Huber 2010). This would most likely occur at low elevation in the southern part of the distribution where *T.hudsonicus* is found, in areas where the maximum temperature of the warmest month is already at the upper limit of what the species can tolerate. Any increase in temperature will therefore not be endurable by the species.

In conclusion, we have defined the thermal niche of *T.hudsonicus* and we have established that it is a critical determinant of the species range. We can expect therefore that temperature will create an enormous selection pressure on the morphology, physiology and genetics as examined in the following chapters 2-4.

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CHAPTER 2

Adaptive geographic variation in external morphology in the American red squirrel, *Tamiasciurus hudsonicus*

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ABSTRACT

Temperature is one of the more substantial environmental constraints that species face and traits associated with adaptation or tolerance to temperature extremes (high or low) are important to investigate. With regards mammals, it is possible to address thermal adaptation through testing two biogeographical rules, Bergmann's rule (which states that mammals will get bigger with reduced temperature) and Allen's rule (which states that appendages will get smaller relative to body size with reduced temperature). These can be tested using gross morphological measurements. Fortunately, longstanding traditions of museum collections and curation, including applying standardized measurements to newly acquired specimens, have led to the accumulation of a vast amount of morphometric and presence data for many North American terrestrial mammals, obtained over a 150 year-period. These data provide an excellent opportunity to consider the extent to which there is an adaptive response in morphology over the wide range of temperatures found on the continent.

Here we studied the American red squirrel, *Tamiasciurus hudsonicus*, a small, extremely widespread and common mammal found inhabiting the coniferous forests of North America. We gathered a dataset of over 9000 specimens from 34 museums from the entire distribution of the species and applied multimodel inference to the standard whole body measurements taken on these specimens.

We found that the largest individuals tended to be found at high elevation and latitudes. We found that appendages (ear, tail and hind foot) varied in size in proportion to the rest of the body. Overall our findings are consistent with Bergmann's rule and thermal adaptation in the *T.hudsonicus*. However, we do not find support for Allen's rule which might indicate selective pressures other than temperature are more important in relation to size of the appendages.

INTRODUCTION

Temperature is an important environmental constraint affecting species of mammal and there are many ways in which they may show trait responses to temperature variation. This includes changes in morphology (e.g. in relation to body or appendage size in cold vs. warm areas or in body size in summer vs. winter; Clauss et al 2013; Griffing 1974; Taylor, Rychlik & Churchfield 2013), behavior (e.g. resting during the warmest part of the day or basking in the sun; Huey et al 2012; Warnecke, Turner & Geiser 2008) and physiology (e.g. increasing heat production, evaporative water loss or changes in blood flow; Scholander et al 1950; Tattersall et al 2012; Johnson, Minson & Kellogg 2014) and many instance of thermal adaptation will undoubtedly involve a combination of strategies (Briscoe et al 2014). In order to best understand the complete picture of thermal adaptation it is important to focus on the relative importance of each of these factors in turn.

Two ecogeographical rules, Bergmann's rule and Allen's rule, have long been used as essentially null models in relation to morphological adaptations to temperature. Bergmann's rule, in its original form, states that endotherms will on average have a larger body size at higher latitudes and elevation (James 1970; Blackburn, Gaston & Lodger 1999; Blackburn & Hawkins 2004). Allen's rule, again first formulated for endotherms, states that appendages (such as the tail and feet) will be smaller relative to body size at higher latitudes and elevation (Fooden & Albrecht 1999; Nudds & Oswald 2007). Both rules follow the same principle in relation to conservation of energy, positing that it is important to have a low surface to volume ratio to minimize heat loss to the environment. However, there are some controversies regarding these rules, including alternative explanations for Bergmann's and Allen's trends and examples where species do not follow these trends. Proposed alternative explanations include e.g. reduction of heat loading in warm areas rather than reduction of heat loss in cold climates (McNab 1979), change in insulation for heat conservation (Scholander 1955, as seen with increased fat storage in moose at higher latitudes; Sand, Cederlund & Danell 1985), the importance of food quality or abundance with the lower productivity of arctic areas e.g. leading to an increased foraging area and a larger body size to move around that (Lindsay 1986; Erlinge 1987; McNab 1963).

Bergmann's and Allen's rules can be examined using museum collections. Since biologists first started systematically collecting for museums there have been standardized methods for a quantitative description of each specimen in hand for accurate comparison between population samples and species. For specimens collected in the US, the measurements for terrestrial mammals include the total body length (measured from the tip of the tail vertebrae to the tip of the nose), the length of the tail (measured from the first to the last vertebrae of the tail), hind foot (measured either from the tip, or the nail, of the longest toe on the right hind foot

to the heel), length of ear (measured from the notch to the most distal part of the right ear) and body mass (Hoffmann et al 2010). Due to this being a longstanding tradition and the long history of museums and museum collections there is a vast amount of data available from over a hundred year period that provides an amazing opportunity to answer basic questions relating to natural variation and evolution (Lister 2011). The vertnet database (Vertnet.org) alone has around 20 million records of specimens, relating to vertebrates. The standard measures taken for terrestrial mammals are clearly critical for taxonomy (Martin et al 2001) and allow particular species of mammal to be defined (e.g. Gündüz et al 2007). At a within-species level, the measurements may help to define major genetic units within species, such as subspecies (e.g. Heaney 1978). The measurements can also be a valuable resource in understanding adaptation in mammals. This particularly applies to thermal adaptation since all of the standardized measurements for mammals may relate to Allen's rule and/or Bergmann's rule.

The American red squirrel, *Tamiasciurus hudsonicus*, a small arboreal mammal, is found throughout the boreal forests of northern North America and extending south along the Rocky Mountains in the west and the Appalachian Mountains in the east (Steele 1998). The species is active all year around, does not hibernate and does not go into torpor (Aleksiuk 1971). It is therefore subjected to very low temperature through the northern parts of its distribution, and at high elevation, and high temperature in lowland areas further south in the distribution. Any patterns of morphological variation supporting either Allen's rule or Bergmann's rule will most likely relate to temperature in this species, because alternative explanations do not appear to apply. The species has very little insulatory fat, does not have conspicuous mammalian competitors that could lead to character displacement (in this case increase or decrease in size) to avoid niche overlap, and does not have greater quantities of food in warmer areas which might

lead to smaller size due to smaller areas of foraging (Rovetto & Ferguson 1971; Steele 1998; Gurnell 1984; Streubel 1968).

Here we make use of the large set of museum records on *T.hudsonicus*, to examine variation in external dimensions over its range (in relation to the geographical features that biogeographical rules are based on), to gain insight into the factors that are of influence, and in particular the role of temperature. We apply multimodel inference to the whole dataset, examining a range of predictor variables recorded for each specimen (relating to sex of specimens, time and location of sampling). The impact of year of sampling is of interest, because this allows us to examine if climate change over recent decades has had an impact on body dimensions. It is well known that despite reasonable stability in temperature over the past millennium, there has been a striking increase during the twentieth century (Mann et al 1999) that is continuing today (climate.nasa.gov). It is also known that these temperature changes impact distributions (e.g. Parmesan et al 1999) and cause extinctions (including of mammals: Gynther, Waller & Leung 2016; Watson 2016), but we have an excellent opportunity to assess whether climate change causes Bergmann-like trends in *T.hudsonicus*, as has been reported in other species (Sheridan & Bickford 2011).

Altogether we make use of records for over 9000 museum specimens collected over a period longer than 150 years, making this one of the largest datasets of this type that has been analyzed. The extensive geographic distribution of *T.hudsonicus* and the vast number of records and the time dimension under conditions of climate change allow us to use this system for a particularly powerful test of Bergmann's and Allen's rules.

MATERIALS AND METHODS

Data collection

The specimen data were gathered using Vertnet (vertnet.org), online museum databases through museum websites, through direct contact with curators and collection managers, or via museum visits.

We used the most commonly reported morphological measurements for our analysis: total body length (TBL; the standard measurement in the USA), head-body length (HBL; the standard measurement in Europe, mammalogists trained in Europe will often record this instead of TBL), tail length (TL), hind foot length (HF), ear length (Ear), body mass (Mass), and two ratios, TL/HBL, and HF/HBL. Where not recorded separately, HBL was calculated by subtracting TL from TBL.

For each specimen there were data available on the following factors: latitude, longitude, elevation, year and sex. These were the factors that were used to construct the models in the multimodel inference.

The total dataset, after filtering for quality, included 9407 individuals collected from 1860-2015 (figure 2.1) from 34 museums (table 2.1).

Table 2.1 – Number and museum origins of specimens used in the study

Museum code	Museum	Number of specimens
AMNH	American Museum of Natural History	783
BYU	Monte L. Bean Life Science Museum	40
CAS	California Academy of Sciences	14
CHAS	Chicago Academy of Sciences	51
CM	Carnegie Museum of Natural History	420
CMNMA	Canadian Museum of Nature	697
CRCM	Charles R Conner Museum	193
CSUC	California State University, Chico Vertebrate Museum	5
CUMV	Cornell University Museum of Vertebrates	287
DMNS	Denver Museum of Nature and Science	146
FMNH	Field Museum of Natural History	304
GMNH	Georgia Museum of Natural History	10
KSTC	Schmidt Museum of Natural History, Emporia State University	2
KU	Kansas University Biodiversity Institute	699
LACM	Natural History Museum of Los Angeles County	30
MCZ	Museum of Comparative Zoology	140
MMNH	Midwest Museum of Natural History (Bell Museum)	61
MSB	Museum of Southwestern Biology	396
NBM	New Brunswick Museum	231
NMMNH	New Mexico Museum of Natural History and Science	5
RBCM	Royal British Columbia Museum	42
	Redpath Museum	14
ROM	Royal Ontario Museum	1101
RSKM	Royal Saskatchewan Museum	26
TTU	Museum of Texas Tech University	6
UAFMC	University of Arkansas Collection Facilities	3
UAM	University of Alaska Museum	646
UBCBBM	University of British Columbia Beaty Biodiversity Collection	202
UCM	University of Colorado Museum of Natural History	169
UIMNH	University of Iowa Museum of Natural History	5
USNM	National Museum of Natural History	2052
UTCM	University of Tennessee at Chattanooga Museum	1
UWBM	University of Washington, Burke Museum	622
WNMU	Western New Mexico University	3
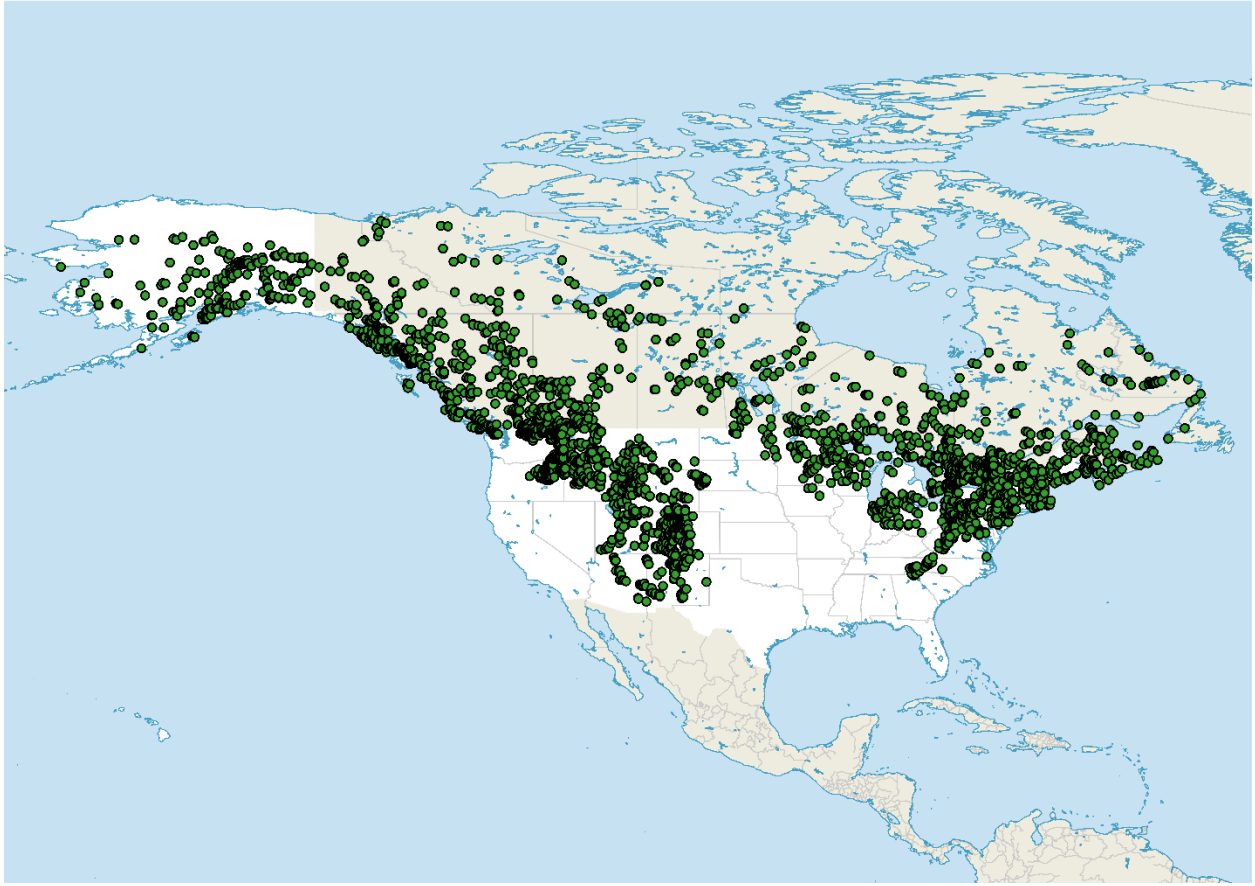
Figure 2.1 – Collection locations for all specimens analyzed in this study

Age determination and quality control

To reduce biases, all juveniles and subadults were excluded from the analysis. For the purpose of this study individuals were simply labelled adults and not divided into subclasses within that classification. Individuals were classified as juveniles until they had reached approximate adult size or sexual maturity. Individuals typically reach full size for all the standard external measurements used in this study by 125 days and full weight shortly thereafter (Layne 1954, based on a study of *T.hudsonicus loquax* in New York). As a first step in determining age we relied on information from collectors or preparators. When lifestage was not listed we used a variety of methods to determine approximate lifestages based on museum notes and personal observation of specimens where applicable (figure 2.2).

All data entries were checked for errors or biases. Individuals may have their tails broken in nature, and TL and TBL of specimens where this could be detected (TL and TBL were outliers while HBL was not), were discarded. Since a defining feature of the species is TL being shorter than HBL, specimens were discarded in cases where TL was larger than HBL and where it was not obvious that HBL and TBL had not been mixed up; HBL was given as TBL in some collections as a common confusion due to difference in standards between countries and hence training of collectors. Since the species has been divided into 25 subspecies based on morphology they were, when applicable, taken into account when looking at outliers.

1. Aged by collector/preparator

Aging not successful

2. Descriptions in notes

Juveniles	Adults
- Testes abdominal[1] - Scrotum inconspicuous - Testes < 7mm[2] - Nipples < 0.5mm	- Testes scrotal - Scrotum pigmented - Scrotum hairless - Testes > 7mm - Nipples darkly colored - Nipples > 1mm - Prominently coiled vagina

Aging not successful

3. Direct observations of specimens

Juveniles	Adults
- Testes abdominal[1] - Scrotum inconspicuous - Nipples < 0.5mm - Incomplete dentition - Lower 3rd molar not worn	- Testes scrotal - Scrotum pigmented - Scrotum hairless - Nipples darkly colored - Nipples > 1mm - P4 permanent - Wear on all teeth

Aging not successful

4. Inferences from collection notes

Adults
- Collection date beteween 01. January and 31. March

Aging not successful

5. Inferences from data and literature

Juveniles	Adults
- 2 st.dev below mean for HF, TL and TBL - Below published lengths for HF, TL and TBL	- TBL > 270mm - TL > 92mm - HF > 35mm

Aging not successful

6. Data not used

Figure 2.2 - Criteria used to classify Tamiasciurus hudsonicus *specimens into age classes. While there are other characteristics that can be utilized, the listed characteristics are the full set that were described in museum notes or were directly observed on the specimens, for the set of specimens used in this study. Based on Mossman, Lawlah & Bradley 1932; Layne 1954; Nellis 1969; Lindsay 1987; Steele 1998.*

[1]Testes are often retracted into the abdomen due to handling; collection method may therefore influence this and for that reason this was evaluated with caution for aging.

[2]From September to November the reproductive organs of adult males are repressed and similar to juveniles; therefore, this was considered when age was determined.

Analysis

For each specimen there were data on latitude, longitude, elevation, year and sex and the impact of these factors on the morphological variables TBL, HBL, TL, HF, Ear, Mass, TL/HBL, and HF/HBL was explored using multimodel inference (Burnham & Anderson 2002). To avoid excessive collinearity among the factors latitude, longitude, elevation, year and sex we determined collinearity using a variance inflation factor (vif) test in R using the car package (R version 1.15.6, R Core Team 2016; Fox et al 2016). Only factors with a vif score within an acceptable range were used for further analysis. From our knowledge of the biology of *T.hudsonicus* we generated 49 candidate models (table S2.1) that we evaluated for each morphological variable. The candidate models were compared using the second-order Akaike information criterion (AIC$_c$) and the best-fitting model was indicated by the lowest AICc value, with each other model i compared to the best using the AICc differences (Δ_i). All models with ΔAICc < 2 were considered, following convention (Burnham & Anderson 2002; Burnham & Anderson 2004). The top models for each variable were selected using the MuMIn package in R (version 1.15.6, R Core Team 2016; Bartoń 2015).

RESULTS

The model most often found to well describe the variation in the skull measurements included all the main factors, and the geographical interactions elevation:longitude, elevation:latitude and longitude:latitude (table 2.2). This model was found to be a good fit for all the morphological variables. However, the spread of best models was fairly even and the lowest Δ_i (and therefore highest W$_i$ i.e. the relative likelihood of the model) were split between six models. Only nine models out of 49 were found to be well supported. The factors found in these well supported models were as follows: latitude, elevation and longitude in all nine models, year in eight and sex

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in six. The interaction between elevation and longitude was found in six models, between

elevation and latitude in eight and between latitude and longitude in seven. Interaction between

year and latitude was only found in one model and was only well supported for one variable

(HF/TBL). For four variables, HBL, TBL, HF and TL, the best models for each have an

accumulated weight of around or above 0.9, i.e. are within the 90% confidence set. While for

TL/TBL only one model was well supported, support for up to four models (in the case of TL)

was found for other variables. Further details of results for each variable can be found in tables

S2.2-S2.9.

Table 2.2 – Well supported models for all morphological variables in the study (as defined by Δi <2) based on the following factors: elevation (Elev), latitude (Lat), longitude (Long), sex and year, with the best models for each variable (Δi = 0) shown in bold

Model	Variable	AICc	Δi	Wi
Elev + Lat + year + sex + Long + Elev:Long + Elev:Lat + Long:Lat	**TL/TBL**	**-35787.7**	**0**	**0.743**
	HF	48651.8	0.1	0.314
	TL	68735.3	0.83	0.185
	Ear	24682.8	0.56	0.313
	HF/TBL	-47012.1	1.53	0.173
	TBL	73595.8	1.56	0.298
	Mass	32642.2	1.95	0.123
	HBL	69487	1.95	0.245
Elev + Lat + year + Long + Elev:Long + Elev:Lat + Long:Lat	**TL**	**68734.5**	**0**	**0.281**
	Ear	**24682.3**	**0**	**0.414**
Elev + Lat + year + sex + Long + Elev:Lat + year:Lat + Long:Lat	HF/TBL	-47012.8	0.84	0.244
Elev + Lat + year + sex + Long + Elev:Long + Elev:Lat	**Mass**	**32640.2**	**0**	**0.325**
	TBL	**73594.3**	**0**	**0.651**
Elev + Lat + year + sex + Long + Lat:Long + Lat:Elev	**HF/TBL**	**-47013.6**	**0**	**0.372**
	HF	48652.2	0.51	0.256
	TL	68735.4	0.9	0.179
Elev + Lat + year + sex + Long + Long:Elev + Long:Lat	**HF**	**48651.7**	**0**	**0.33**
Elev + Lat + year + Long + Elev:Long + Elev:Lat	Mass	32640.6	0.36	0.272
Elev + Lat + year + Long + Lat:Long + Lat:Elev	TL	68734.6	0.07	0.271
Elev + Lat + sex + Long + Elev:Long + Elev:Lat + Long:Lat	**HBL**	**69485.1**	**0**	**0.651**

* AICc = second-order Akaike information criterion; Δ_i difference between a model and the highest scoring mode; W_i = model weight, the relative likelihood of a model.

Considering the geographical interactions, all of which are found to be important for all variables, figures 2.3-2.5 show a clear pattern where large individuals are found at higher elevation, low and high latitudes (but less so in between), and at intermediate longitude.
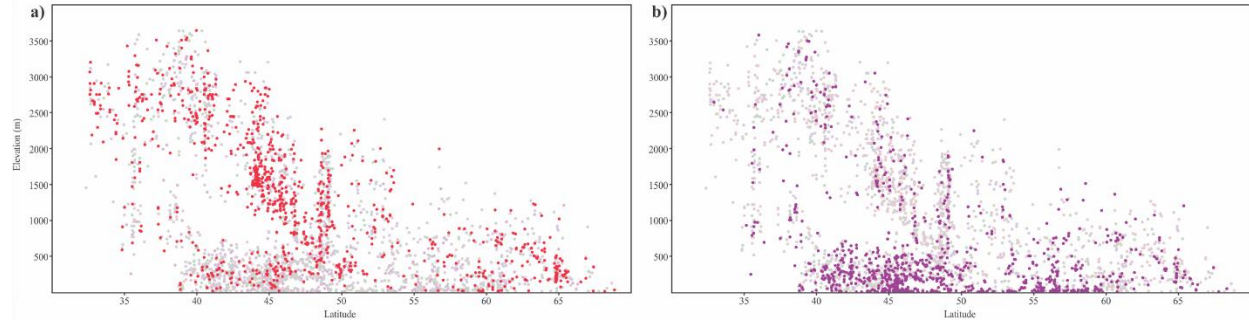
Figure 2.3 – Interaction between latitude (degrees) and elevation (m) for TBL; a) longest 20% of specimens, b) shortest 20% of specimens.
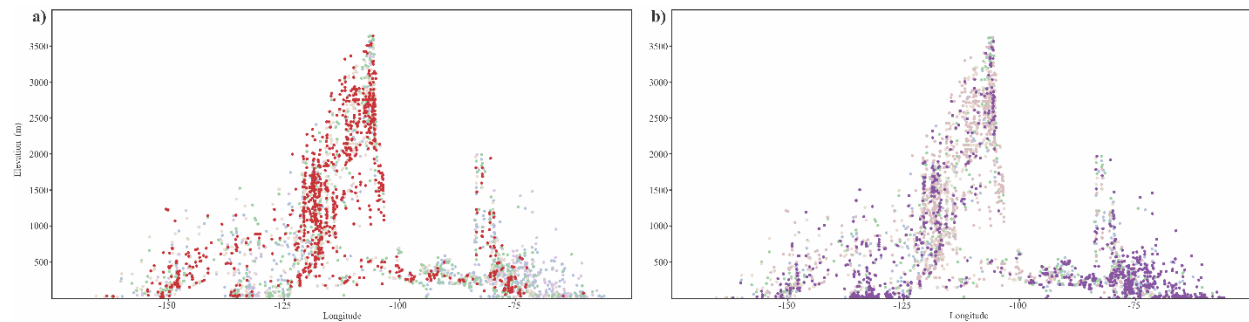
Figure 2.4 – Interaction between longitude (degrees) and elevation (m) for TBL; a) longest 20% of specimens, b) shortest 20% of specimens.
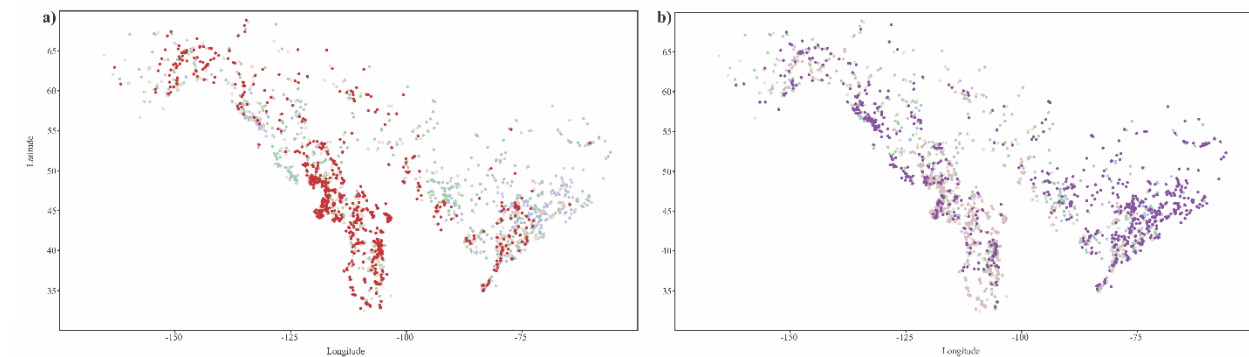
Figure 2.5 – Interaction between latitude (degrees) and longitude (degrees) for TBL; a) longest 20% of specimens, b) shortest 20% of specimens.

Weak relationships were found between the morphological variables and year (e.g. TBL: figure 2.6).
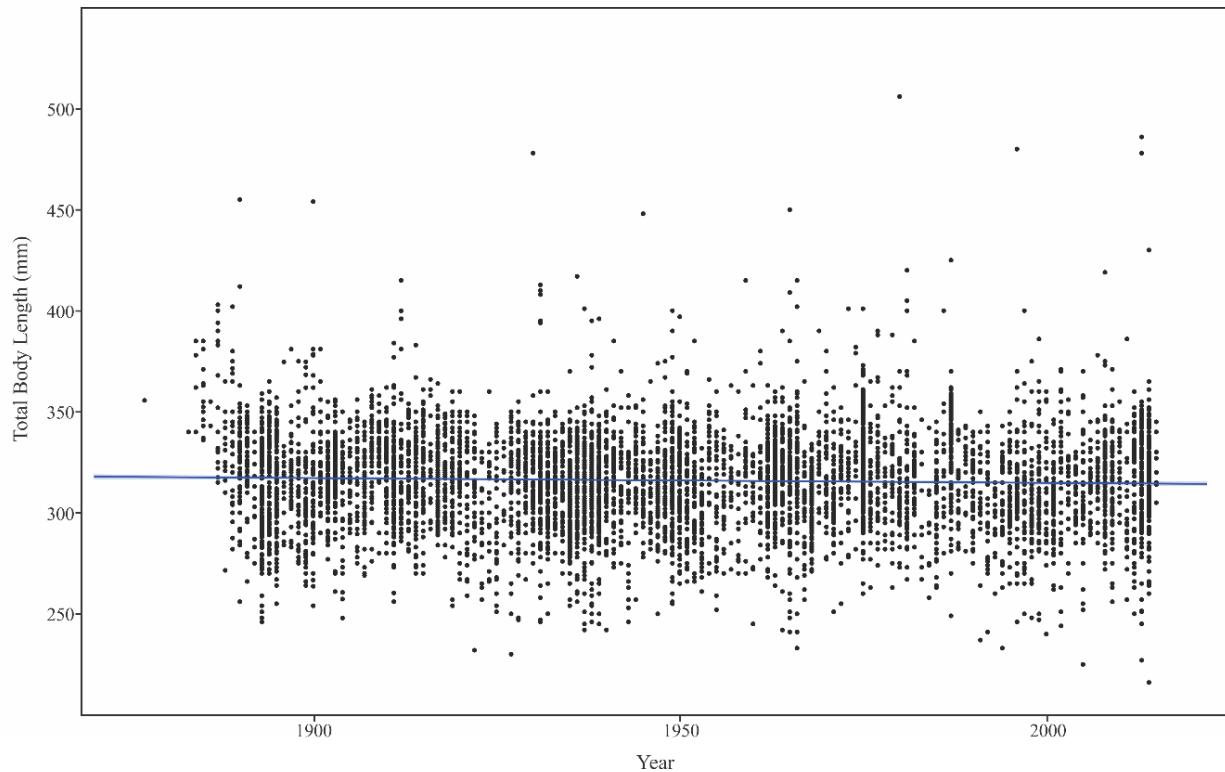
Figure 2.6 – Changes in total body length (TBL) through time, with a fitted linear regression

Males were found to be larger than females for all variables, except the ratios, and this size difference was not limited to specific parts of the distribution (table 2.3).

On examination of bivariate plots between morphological variables and main factors we found weak or no relationships in most cases, as indicated by the R-squared values (table 2.4). If Allen's rule was supported we would expect HF, TL, HF/HBL and TL/HBL to decrease with increased elevation and latitude. If Bergmann's rule was supported we would expect all variables to increase with elevation and latitude. All morphological variables but one showed positive relationships with elevation (TL/HBL showed no relationship). Similarly variables other than HF/HBL (no relationship) and TL/HBL (positive) had negative relationships with longitude.

TBL, TL, HF, Ear and TL/HBL had negative relationships with latitude while HF/HBL and

Mass had positive relationships with latitude and HBL showed no relationship. As already

indicated, all relationships between morphological variables and year were very weak or not

apparent at all (HF and HF/HBL). TBL, TL, HBL, TL/HBL and Ear had weak negative

relationships with year while Mass had a weak positive relationship with year.

Table 2.3 – Means and standard deviations for morphological variables in males and females

	Female	Male
TBL (mm)	315.03 ± 21.98	316.97 ± 23.89
HBL (mm)	190.06 ± 16.29	191.59 ± 17.53
Mass (g)	198.97 ± 44.37	200.84 ± 43.29
TL (mm)	124.77 ± 11.88	124.99 ± 12.54
HF (mm)	48.07 ± 3.70	48.52 ± 4.05
Ear (mm)	23.52 ± 3.93	23.74 ± 4.03
TL/HBL	0.40 ± 0.03	0.40 ± 0.03
HF/HBL	0.15 ± 0.01	0.15 ± 0.01

Table 2.4 – R-squared values for linear regressions of morphological variables on the factors Elevation, Latitude (South to North), Longitude (West to East), and Year. Arrows show direction of regression with ↑ indicating a positive regression and ↓ a negative regression.

	Elevation	Latitude	Longitude	Year
TBL (mm)	$R^2 = 0.101\ (\uparrow)$	$R^2 = 0.005\ (\downarrow)$	$R^2 = 0.076\ (\downarrow)$	$R^2 = 0.001\ (\downarrow)$
HBL (mm)	$R^2 = 0.070\ (\uparrow)$	$R^2 = 0.000$	$R^2 = 0.082\ (\downarrow)$	$R^2 = 0.003\ (\downarrow)$
Mass (g)	$R^2 = 0.097\ (\uparrow)$	$R^2 = 0.002\ (\uparrow)$	$R^2 = 0.103\ (\downarrow)$	$R^2 = 0.015\ (\uparrow)$
TL (mm)	$R^2 = 0.064\ (\uparrow)$	$R^2 = 0.014\ (\downarrow)$	$R^2 = 0.021\ (\downarrow)$	$R^2 = 0.015\ (\downarrow)$
HF (mm)	$R^2 = 0.070\ (\uparrow)$	$R^2 = 0.001\ (\downarrow)$	$R^2 = 0.124\ (\downarrow)$	$R^2 = 0.000$
Ear (mm)	$R^2 = 0.043\ (\uparrow)$	$R^2 = 0.014\ (\downarrow)$	$R^2 = 0.007\ (\downarrow)$	$R^2 = 0.002\ (\downarrow)$
TL/HBL	$R^2 = 0.000$	$R^2 = 0.009\ (\downarrow)$	$R^2 = 0.013\ (\uparrow)$	$R^2 = 0.023\ (\downarrow)$
HF/HBL	$R^2 = 0.003\ (\uparrow)$	$R^2 = 0.007\ (\uparrow)$	$R^2 = 0.000$	$R^2 = 0.000$

DISCUSSION

T.hudsonicus has a very large geographic range in North America and the museum collecting for

the species has been remarkably comprehensive over the range (figure 2.1). There has also been

consistent sampling from the late 1800s to the early 2000s (figure 2.6). Given the occurrence of

over 9000 records and the varied topography and geographic variation in temperature regimes,

this system is an excellent one for examining the environmental determinants of body size and appendage size. Therefore, the fit of data to Bergmann's and Allen's rule can be examined as part of a wider consideration of thermal adaptations shown by *T.hudsonicus.*

In the multimodel inference the factors that we included in the models were elevation, longitude, latitude, sex and year; elevation, latitude and longitude being the original variables used when the ecogeographical rules were established. All were well represented in the best supported models. While elevation, longitude and latitude do not show strong trends in bivariate analyses the interactions between these geographical factors show clear trends (figures 2.3 – 2.5). All measurements show that body size increases with increased elevation although at higher latitudes there are larger individuals at lower elevation as well. This trend provides support for Bergmann's rule. Due to the nature of the distribution the effect of latitude is not as clear. This can be seen when looking at figures 2.3 & 2.5. This is since the tree line is lower in the northern part of the range and the species cannot be found as high up both for that reason and due to extreme climate at high elevation in the far north; similarly, the species is only found in mountainous areas in the south due to restriction of habitat and climate (chapter 1). Specimens collected in the Rocky Mountains and in the southern part of the Appalachians are mostly larger than average. In the west this can be seen as far north as Canada while in the east this is mainly seen in Georgia, Tennessee, North Carolina and West Virginia. This means that while the majority of the distribution of the species in the east comprise relatively small squirrels, at corresponding latitudes in the west they are very large. Where the distribution is in the middle of North America the squirrels are again very small, leading to a considerable latitudinal belt around 55N where squirrels are relatively small. The latitudinal effects therefore appear to be due to the impact of elevation. Overall these findings seem to provide support for Bergmann's

rule in that *T.hudsonicus* at high latitude and high elevations tend to be larger. There is little support for Allen's rule. The three 'appendages' (ear, hindfoot and tail) all show increase in size (both HF and TL increase proportionately to TBL) up an elevational gradient, contrary to what the rule would predict. While there is some indication that there might be reduction in the size of the appendages with latitude, this is unclear and may be due to confounding effects.

Sex proved to be an important factor in the majority of the models, and for all variables. This was perhaps not to be expected. While many mammals show sexual dimorphism with one or the other sex being bigger this has not been systematically reported for *T.hudsonicus* (Steele 1998; Vernes 2004). The consensus has been that the species is not sexually dimorphic and where dimorphism is found it is limited to certain geographic regions and subspecies (Boutin & Larsen 1993; Layne 1954). Some authors have found specific measurements, namely minor skull features, to differ between the sexes with males on average being larger, although not in all cases (Lindsay 1987). Here we find that males are larger than females when looking at all of variables except the ratios which did not differ between the sexes. We did not find sex to be interacting with any geographical predictor and therefore conclude that the pattern is not limited to certain areas and not a confounding effect in our dataset.

Regarding the Bergmann trend of greater size with northernmost latitudes and high elevations in the south in *T.hudsonicus*, these results indicate a need to be cautious in interpreting latitudinal trends in body size. The norm is to assess Bergmann's rule by analysis of body size against latitude (Ashton, Tracy & de Queiroz 2000) and therefore an increase in body size towards lower latitudes is seen as a converse to Bergmann's rule. However, if the localities at lower latitudes are at a higher elevation, then, in terms of thermal conservation, they may actually be following Bergmann's rule, because localities at high elevation can be exposed to

very low temperatures. As we have indicated, *T.hudsonicus* populations at low elevation in the eastern US are smaller than high elevation populations in the western US (figure 2.5). A European example of a species that shows increasing size with lower latitudes is the pygmy shrew (*Sorex minutus*) (Vega et al 2010, 2016); like *T.hudsonicus* it follows mountain chains (such as the Apennines) in the southern part of its range, and therefore may be showing converse Bergmann's rule on the basis of latitude, but Bergmann's rule on the basis of temperature.

Given the evidence for Bergmann's rule with geography in *T.hudsonicus*, it might appear surprising that there is not a Bergmann trend over time in the species; with decreasing body size with increasing temperature during the twentieth century, as seen in other mammals (Post et al 1997). This requires further investigation. The data on *T.hudsonicus* is obtained range wide. One possibility is that *T.hudsonicus* have remained the same size because the whole distribution has moved northwards or up an elevation gradient.

While there is qualified support for Bergmann's rule in *T.hudsonicus*, Allen's rule is not supported. Although it would seem to be advantageous to have smaller appendages under cold conditions, there may be constraints which do not allow this type of morphological change. For instance, tails have many functions (Hickman 1979) and for a very fast running and jumping arboreal species like *T.hudsonicus* (Layne 1954), tails of a particular length are likely to be essential for balance. Tails in *T.hudsonicus* are also used as part of thermoregulation under cold conditions, contributing to insulation (Pauls 1978). It is interesting that larger body size is allowable at higher latitudes and elevations in *T.hudsonicus*, given that such a size increase may hinder their rapid movements. However, the thermoregulatory advantages may outweigh any loss in agility. Further studies would be worthwhile to investigate these points.

On a final note, it is worth emphasizing that while museum datasets are of immense value it is essential to be cautious in making use of the data and results. To illustrate this we can look at the impact of year which we might have expected to indicate changes in morphology in response to climate change, with size reduction due to increased temperature. However, all relationships between morphological variables and year are very weak and may be subject to biases. When looking at TBL and TL the apparent decrease in size with time seems to be driven by the large size of specimens collected before 1890 (figure 2.6). A large number of those samples are from New York State, collected by one person. The average length of these individuals is much greater than the average length in later years, which stays constant throughout the twentieth century. As an example, the average TBL of the early New York squirrels is around 30mm longer than the average length for any ten year time period after 1900 and much greater than any published values for *T.hudsonicus loquax* (the subspecies found in the region; Layne 1954; Whitaker & Hamilton 1998). While it is impossible to say exactly why these squirrels are this big, it is likely that some measurement error is involved. Given that HBL is not impacted in the same way as TBL and TL it may be suggested that the collector included the tail hairs, which extend beyond the tip of the tail vertebrae, in his measurements. Increase in body mass is also likely due to sampling bias. Measurement of body mass was not as easily obtained as length measurements for researchers early on and between 1846 and 1906 for only 25 samples was body mass recorded, this is out of 1308 samples in our dataset from that time period. When these early samples were removed, thereby limiting the analysis to the period where body mass was frequently measured, there was no relationship between body mass and year.

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SUPPLEMENTARY MATERIAL 2

Table S2.1 – Models evaluated for the study

Model Number	Model factors
Model 1	Elevation + Latitude + Year + Sex + Longitude
Model 2	Elevation + Year + Sex + Longitude
Model 3	Elevation + Year + Longitude + Latitude
Model 4	Elevation + Latitude + Sex + Longitude
Model 5	Elevation + Latitude + Longitude
Model 6	Elevation + Year + Latitude
Model 7	Elevation + Year + Longitude
Model 8	Elevation + Longitude
Model 9	Latitude + Longitude
Model 10	Latitude + Elevation
Model 11	Elevation + Year + Latitude + Longitude + Year:Elevation
Model 12	Elevation + Year + Latitude + Longitude + Year:Longitude
Model 13	Elevation + Year + Latitude + Longitude + Year:Latitude
Model 14	Elevation + Year + Latitude + Longitude + Elevation:Longitude
Model 15	Elevation + Year + Longitude + Latitude + Latitude:Longitude
Model 16	Elevation + Year + Longitude + Latitude + Elevation:Latitude
Model 17	Elevation + Latitude + Longitude + Elevation:Longitude
Model 18	Elevation + Latitude + Longitude + Elevation:Latitude
Model 19	Elevation + Latitude + Longitude + Longitude:Latitude
Model 20	Elevation + Year + Longitude + Elevation:Longitude
Model 21	Latitude + Year + Longitude + Latitude:Longitude
Model 22	Latitude + Year + Elevation + Latitude:Elevation
Model 23	Elevation + Longitude + Elevation:Longitude
Model 24	Latitude + Longitude + Latitude:Longitude
Model 25	Latitude + Elevation + Latitude:Elevation
Model 26	Elevation + Latitude + Year + Sex + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 27	Elevation + Latitude + Year + Sex + Longitude + Elevation:Year + Year:Latitude
Model 28	Elevation + Latitude + Year + Sex + Longitude + Elevation:Latitude + Year:Latitude + Longitude:Latitude
Model 29	Elevation + Latitude + Sex + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 30	Elevation + Latitude + Year + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 31	Elevation + Latitude + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 32	Elevation + Year + Sex + Latitude + Longitude + Sex:Elevation
Model 33	Elevation + Year + Sex + Latitude + Longitude + Sex:Latitude
Model 34	Elevation + Year + Sex + Latitude + Longitude + Sex:Longitude

Model 35	Elevation + Sex + Latitude + Longitude + Sex:Elevation
Model 36	Elevation + Sex + Latitude + Longitude + Sex:Latitude
Model 37	Elevation + Sex + Latitude + Longitude + Sex:Longitude
Model 38	Elevation + Latitude + Year + Sex + Longitude + Elevation:Longitude + Elevation:Latitude
Model 39	Elevation + Latitude + Sex + Longitude + Elevation:Longitude + Elevation:Latitude
Model 40	Elevation + Latitude + Year + Longitude + Elevation:Longitude + Elevation:Latitude
Model 41	Elevation + Latitude + Longitude + Elevation:Longitude + Elevation:Latitude
Model 42	Elevation + Latitude + Year + Sex + Longitude + Latitude:Longitude + Latitude:Elevation
Model 43	Elevation + Latitude + Sex + Longitude + Latitude:Longitude + Latitude:Elevation
Model 44	Elevation + Latitude + Year + Longitude + Latitude:Longitude + Latitude:Elevation
Model 45	Elevation + Latitude + Longitude + Latitude:Longitude + Latitude:Elevation
Model 46	Elevation + Latitude + Year + Sex + Longitude + Longitude:Elevation + Longitude:Latitude
Model 47	Elevation + Latitude + Sex + Longitude + Longitude:Elevation + Longitude:Latitude
Model 48	Elevation + Latitude + Year + Longitude + Longitude:Elevation + Longitude:Latitude
Model 49	Elevation + Latitude + Longitude + Longitude:Elevation + Longitude:Latitude

Table S2.2 – Results from model selection for TBL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	sex	Yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
38	427.1	-0.0185	-0.8972	-0.4010	+	-0.0592				-0.0001		0.0003				9	-36788.11	73594.30	0.00	0.65
26	419.8	-0.0178	-0.7731	-0.4576	+	-0.0586				-0.0001	0.0011	0.0003				10	-36787.89	73595.80	1.56	0.30
42	409.4	-0.0114	-0.6506	-0.5426	+	-0.0577						0.0023	0.0003			9	-36791.05	73600.10	5.87	0.04
28	359.1	-0.0112	0.3732	-0.5256	+	-0.0310		-0.0005			0.0020	0.0003				10	-36790.81	73601.70	7.40	0.02
40	429.3	-0.0186	-0.9000	-0.4003		-0.0596				-0.0001		0.0003				8	-36799.52	73615.10	20.81	0.00
30	422	-0.0179	-0.7767	-0.4566		-0.0591				-0.0001	0.0011	0.0003				9	-36799.30	73616.60	22.38	0.00
44	411.7	-0.0115	-0.6541	-0.5417		-0.0581						0.0023	0.0003			8	-36802.46	73620.90	26.68	0.00
16	427	-0.0121	-0.9219	-0.4248		-0.0593						0.0004				7	-36803.55	73621.10	26.87	0.00
46	412	-0.0156	-0.5671	-0.4478	+	-0.0578				-0.0002	0.0015					9	-36802.52	73623.10	28.80	0.00
27	573.1	-0.0810	-2.3410	-0.4141	+	-0.1405	0.0000		0.0009							9	-36809.58	73637.20	42.94	0.00
14	424.5	-0.0168	-0.7421	-0.3664		-0.0590				-0.0002						7	-36814.66	73643.30	49.08	0.00
48	414.2	-0.0158	-0.5684	-0.4466		-0.0582				-0.0002	0.0015					8	-36814.22	73644.50	50.21	0.00
11	484.4	-0.0724	-0.6684	-0.4148		-0.0943	0.0000									7	-36821.75	73657.50	63.25	0.00
1	412.1	0.0033	-0.6806	-0.4103	+	-0.0572										7	-36825.82	73665.70	71.40	0.00
33	411	0.0033	-0.6486	-0.4106	+	-0.0574								+		8	-36825.22	73666.50	72.21	0.00
32	412.2	0.0032	-0.6808	-0.4103	+	-0.0572							+			8	-36825.75	73667.50	73.26	0.00
34	411.8	0.0033	-0.6808	-0.4133	+	-0.0572									+	8	-36825.78	73667.60	73.32	0.00
29	297.8	-0.0156	-0.5806	-0.5313	+					-0.0001	0.0029	0.0003				9	-36825.73	73669.50	75.24	0.00
39	314.4	-0.0175	-0.9090	-0.3810	+					-0.0001		0.0003				8	-36827.28	73670.60	76.34	0.00
43	290.9	-0.0105	-0.4834	-0.5996	+						0.0038	0.0003				8	-36827.80	73671.60	77.37	0.00
15	381.1	0.0030	-0.1051	-0.6728		-0.0552					0.0052					7	-36831.96	73677.90	83.69	0.00
13	222	0.0033	3.2050	-0.4143		0.0408		-0.0020								7	-36834.14	73682.30	88.04	0.00
3	414.2	0.0033	-0.6813	-0.4095		-0.0576										6	-36837.74	73687.50	93.25	0.00
12	335.2	0.0033	-0.6763	-1.1140		-0.0171		0.0004								7	-36836.87	73687.80	93.51	0.00
31	299.1	-0.0158	-0.5829	-0.5308						-0.0001	0.0029	0.0003				8	-36837.60	73691.20	96.97	0.00
41	315.8	-0.0177	-0.9120	-0.3802						-0.0001		0.0003				7	-36839.15	73692.30	98.07	0.00
45	292.3	-0.0106	-0.4857	-0.5990							0.0038	0.0003				7	-36839.66	73693.30	99.08	0.00
47	291.9	-0.0136	-0.3845	-0.5209	+					-0.0002	0.0033					8	-36839.21	73694.40	100.18	0.00
18	314.2	-0.0115	-0.9326	-0.4034								0.0003				6	-36842.71	73697.40	103.19	0.00
49	293.2	-0.0137	-0.3845	-0.5202						-0.0002	0.0033					7	-36851.36	73716.70	122.47	0.00
17	312.3	-0.0159	-0.7580	-0.3474						-0.0002						6	-36853.38	73718.80	124.52	0.00
21	374.5		-0.0940	-0.8923		-0.0552					0.0080					6	-36856.59	73725.20	130.94	0.00
4	303.6	0.0034	-0.6984	-0.3902	+											6	-36862.11	73736.20	141.98	0.00
36	302.4	0.0034	-0.6744	-0.3903	+									+		7	-36861.78	73737.60	143.31	0.00
35	303.7	0.0034	-0.6985	-0.3901	+								+			7	-36862.06	73738.10	143.89	0.00
37	303.4	0.0034	-0.6985	-0.3918	+										+	7	-36862.10	73738.20	143.96	0.00
19	269.2	0.0030	0.0223	-0.7196							0.0065					6	-36865.41	73742.80	148.58	0.00
5	304.9	0.0034	-0.6992	-0.3892												5	-36874.47	73759.00	164.70	0.00
24	262.7		0.0333	-0.9387							0.0093					5	-36889.77	73789.60	195.30	0.00
2	398.1	0.0070		-0.2542	+	-0.0600										6	-36894.99	73802.00	207.73	0.00

20	406.7	-0.0071		-0.2126		-0.0617				-0.0001					6	-36895.06	73802.10	207.89	0.00
7	400.3	0.0070		-0.2532		-0.0605									5	-36906.85	73823.70	229.46	0.00
9	316		-1.0750	-0.4842											4	-36909.61	73827.20	232.97	0.00
23	288.9	-0.0059		-0.1892						-0.0001					5	-36936.53	73883.10	288.82	0.00
8	285	0.0072		-0.2275											4	-36946.68	73901.40	307.12	0.00
22	360.6	-0.0001	0.2959			-0.0351						0.0002			6	-37110.64	74233.30	639.05	0.00
25	294.4	-0.0001	0.2524									0.0002			5	-37123.73	74257.50	663.21	0.00
6	353.6	0.0100	0.4284			-0.0346									5	-37124.92	74259.80	665.59	0.00
10	288.5	0.0099	0.3838												4	-37137.56	74283.10	688.88	0.00

Table S2.3 – Results from model selection for HBL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
29	160.3	-0.0153	0.0309	-0.4557	+					-0.0001	0.0043	0.0001				9	-34733.53	69485.10	0.00	0.65
26	162.7	-0.0154	0.0269	-0.4542	+	-0.0012				-0.0001	0.0043	0.0001				10	-34733.51	69487.00	1.95	0.25
47	158.2	-0.0146	0.1014	-0.4516	+					-0.0002	0.0044					8	-34736.74	69489.50	4.40	0.07
46	160.2	-0.0146	0.0982	-0.4504	+	-0.0009				-0.0002	0.0044					9	-34736.72	69491.50	6.37	0.03
39	185.3	-0.0181	-0.4608	-0.2304	+					-0.0001		0.0001				8	-34739.80	69495.60	10.53	0.00
38	191.8	-0.0182	-0.4601	-0.2316	+	-0.0034				-0.0001		0.0001				9	-34739.56	69497.10	12.06	0.00
43	148.1	-0.0062	0.2046	-0.5774	+						0.0060	0.0002				8	-34745.31	69506.60	21.54	0.00
31	161.5	-0.0154	0.0270	-0.4545						-0.0001	0.0043	0.0001				8	-34746.14	69508.30	23.20	0.00
42	147.6	-0.0062	0.2054	-0.5776	+	0.0003					0.0060	0.0002				9	-34745.31	69508.60	23.55	0.00
28	109.2	-0.0061	0.9848	-0.5645	+	0.0207			-0.0004		0.0058	0.0002				10	-34745.06	69510.10	25.06	0.00
30	164.7	-0.0155	0.0217	-0.4524		-0.0015				-0.0001	0.0042	0.0001				9	-34746.09	69510.20	25.11	0.00
49	159.3	-0.0147	0.0999	-0.4502						-0.0002	0.0044					7	-34749.56	69513.10	28.03	0.00
48	162.1	-0.0147	0.0955	-0.4484		-0.0013				-0.0002	0.0044					8	-34749.52	69515.10	29.97	0.00
41	186.3	-0.0182	-0.4630	-0.2299						-0.0001		0.0001				7	-34752.34	69518.70	33.61	0.00
40	193.6	-0.0183	-0.4623	-0.2312		-0.0038				-0.0001		0.0001				8	-34752.05	69520.10	35.03	0.00
17	184.9	-0.0175	-0.4033	-0.2172						-0.0002						6	-34756.20	69524.40	39.33	0.00
14	191.9	-0.0176	-0.4024	-0.2184		-0.0037				-0.0002						7	-34755.93	69525.90	40.79	0.00
45	149.3	-0.0063	0.2001	-0.5757							0.0060	0.0002				7	-34757.79	69529.60	44.50	0.00
44	149.6	-0.0063	0.1997	-0.5755		-0.0001					0.0060	0.0002				8	-34757.79	69531.60	46.50	0.00
18	183.6	-0.0076	-0.4981	-0.2697								0.0002				6	-34771.21	69554.40	69.34	0.00
16	189.7	-0.0076	-0.4976	-0.2708		-0.0032						0.0002				7	-34771.00	69556.00	70.93	0.00
19	135.6	0.0018	0.5020	-0.6470							0.0076					6	-34774.28	69560.60	75.47	0.00
15	132.8	0.0018	0.5054	-0.6482		0.0014					0.0076					7	-34774.24	69562.50	77.40	0.00
27	154.1	-0.0369	0.9183	-0.2659	+	0.0110	0.0000		-0.0006							9	-34772.69	69563.40	78.31	0.00
36	174.7	0.0024	-0.3093	-0.2610	+									+		7	-34782.60	69579.20	94.11	0.00
4	176.3	0.0024	-0.3419	-0.2608	+											6	-34783.71	69579.40	94.33	0.00
35	176.5	0.0022	-0.3424	-0.2608	+								+			7	-34783.36	69580.70	95.64	0.00
33	178.9	0.0024	-0.3084	-0.2618	+	-0.0022								+		8	-34782.50	69581.00	95.93	0.00
1	180.1	0.0024	-0.3414	-0.2615	+	-0.0020										7	-34783.63	69581.30	96.18	0.00
37	176.1	0.0024	-0.3420	-0.2633	+										+	7	-34783.65	69581.30	96.23	0.00
32	180.3	0.0022	-0.3419	-0.2615	+	-0.0020							+			8	-34783.28	69582.60	97.49	0.00
34	179.8	0.0024	-0.3415	-0.2640	+	-0.0020									+	8	-34783.57	69583.20	98.08	0.00
11	224.5	-0.0441	-0.3345	-0.2642		-0.0247	0.0000									7	-34785.88	69585.80	100.69	0.00
24	131.4		0.5113	-0.7835							0.0093					5	-34791.10	69592.20	107.12	0.00
21	128.3		0.5152	-0.7849		0.0016					0.0093					6	-34791.05	69594.10	109.02	0.00
13	-10.45	0.0023	3.5370	-0.2657		0.0961			-0.0020							7	-34790.10	69594.20	109.12	0.00
12	86.57	0.0024	-0.3354	-1.1090		0.0464		0.0004								7	-34794.24	69602.50	117.40	0.00
5	177.4	0.0024	-0.3422	-0.2600												5	-34796.61	69603.20	118.13	0.00
3	181.8	0.0024	-0.3415	-0.2608		-0.0023										6	-34796.50	69605.00	119.92	0.00

23	172.5	-0.0122		-0.1332						-0.0002						5	-34798.75	69607.50	122.41	0.00
20	181.9	-0.0123		-0.1351		-0.0049				-0.0002						6	-34798.26	69608.50	123.45	0.00
2	172.7	0.0043		-0.1833	+	-0.0032										6	-34814.68	69641.40	156.28	0.00
9	185.1		-0.6037	-0.3262												4	-34827.33	69662.70	177.58	0.00
8	167.6	0.0043		-0.1811												4	-34827.73	69663.50	178.37	0.00
7	174.5	0.0043		-0.1826		-0.0036										5	-34827.47	69665.00	179.86	0.00
22	147.2	0.0001	0.2813			0.0122							0.0002			6	-34995.27	70002.60	517.47	0.00
25	170.1	0.0001	0.2962										0.0002			5	-34998.13	70006.30	521.17	0.00
6	142.9	0.0067	0.3672			0.0124										5	-35006.18	70022.40	537.27	0.00
10	166.2	0.0067	0.3829													4	-35009.13	70026.30	541.17	0.00

Table S2.4 – Results from model selection for TL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
30	261.70	-0.0017	-0.8802	0.0431		-0.0577				0.0000	-0.0041	0.0001				9	-34358.24	68734.50	0.00	0.28
44	264.80	-0.0037	-0.9168	0.0693		-0.0580					-0.0044	0.0001				8	-34359.27	68734.60	0.07	0.27
26	261.40	-0.0017	-0.8794	0.0428	+	-0.0577				0.0000	-0.0041	0.0001				10	-34357.65	68735.30	0.83	0.19
42	264.50	-0.0037	-0.9160	0.0690	+	-0.0579					-0.0044	0.0001				9	-34358.68	68735.40	0.90	0.18
28	236.50	-0.0036	-0.3468	0.0784	+	-0.0431			-0.0003		-0.0046	0.0001				10	-34358.45	68736.90	2.43	0.08
40	233.90	0.0009	-0.4210	-0.1719		-0.0555				0.0001		0.0001				8	-34368.95	68753.90	19.42	0.00
38	233.70	0.0009	-0.4207	-0.1720	+	-0.0555				0.0001		0.0001				9	-34368.34	68754.70	20.21	0.00
15	254.90	0.0015	-0.7362	0.0271		-0.0571					-0.0035					7	-34371.94	68757.90	23.41	0.00
48	258.40	-0.0006	-0.7851	0.0516		-0.0574				0.0000	-0.0039					8	-34371.19	68758.40	23.91	0.00
46	258.10	-0.0006	-0.7846	0.0513	+	-0.0574				0.0000	-0.0039					9	-34370.52	68759.10	24.57	0.00
16	235.30	-0.0029	-0.4092	-0.1581		-0.0557						0.0001				7	-34373.25	68760.50	26.03	0.00
27	360.60	-0.0273	-2.4170	-0.1542	+	-0.1215	0.0000		0.0011							9	-34375.63	68769.30	34.78	0.00
11	247.90	-0.0162	-0.3441	-0.1558		-0.0636	0.0000									7	-34378.50	68771.00	36.52	0.00
3	232.10	0.0012	-0.3470	-0.1546		-0.0554										6	-34381.19	68774.40	39.91	0.00
1	231.80	0.0012	-0.3469	-0.1547	+	-0.0553										7	-34380.51	68775.00	40.54	0.00
14	231.80	0.0019	-0.3450	-0.1560		-0.0553				0.0000						7	-34381.11	68776.20	41.75	0.00
13	245.30	0.0012	-0.6142	-0.1542		-0.0621			0.0001							7	-34381.14	68776.30	41.80	0.00
12	224.20	0.0012	-0.3465	-0.2254		-0.0513		0.0000								7	-34381.16	68776.30	41.85	0.00
32	231.70	0.0013	-0.3467	-0.1547	+	-0.0553							+			8	-34380.33	68776.70	42.19	0.00
33	232.10	0.0012	-0.3539	-0.1546	+	-0.0552								+		8	-34380.42	68776.80	42.36	0.00
34	232.00	0.0012	-0.3468	-0.1524	+	-0.0553									+	8	-34380.42	68776.90	42.37	0.00
21	250.40		-0.7223	-0.0866		-0.0567					-0.0021					6	-34392.58	68797.20	62.68	0.00
20	223.60	0.0064		-0.0855		-0.0567				0.0000						6	-34440.14	68892.30	157.80	0.00
7	225.00	0.0031		-0.0760		-0.0569										5	-34442.26	68894.50	160.04	0.00
2	224.70	0.0031		-0.0762	+	-0.0568										6	-34441.57	68895.10	160.66	0.00
31	141.90	0.0001	-0.6978	-0.0296						0.0000	-0.0023	0.0001				8	-34476.62	68969.20	234.76	0.00
29	141.70	0.0001	-0.6970	-0.0299	+					0.0000	-0.0023	0.0001				9	-34475.77	68969.60	235.07	0.00
45	146.00	-0.0030	-0.7542	0.0113							-0.0029	0.0001				7	-34479.12	68972.30	237.77	0.00
43	145.70	-0.0030	-0.7534	0.0110	+						-0.0029	0.0001				8	-34478.27	68972.60	238.07	0.00
41	128.40	0.0016	-0.4344	-0.1532						0.0001		0.0001				7	-34480.10	68974.20	239.72	0.00
39	128.20	0.0016	-0.4340	-0.1533	+					0.0001		0.0001				8	-34479.24	68974.50	240.01	0.00
18	129.50	-0.0025	-0.4216	-0.1381								0.0001				6	-34485.04	68982.10	247.60	0.00
19	139.00	0.0014	-0.6017	-0.0241							-0.0022					6	-34488.14	68988.30	253.81	0.00
49	139.40	0.0012	-0.6078	-0.0211						0.0000	-0.0022					7	-34488.13	68990.30	255.79	0.00
47	139.10	0.0012	-0.6074	-0.0214	+					0.0000	-0.0022					8	-34487.19	68990.40	255.91	0.00
5	127.20	0.0013	-0.3646	-0.1350												5	-34491.52	68993.10	258.56	0.00
4	127.00	0.0013	-0.3645	-0.1352	+											6	-34490.58	68993.20	258.69	0.00
36	127.70	0.0013	-0.3796	-0.1351	+									+		7	-34490.16	68994.30	259.85	0.00
17	126.70	0.0025	-0.3608	-0.1378						0.0000						6	-34491.22	68994.50	259.96	0.00
35	126.90	0.0014	-0.3643	-0.1352	+								+			7	-34490.34	68994.70	260.20	0.00

37	127.30	0.0013	-0.3644	-0.1316	+									+	7	-34490.38	68994.80	260.28	0.00
9	131.10		-0.5005	-0.1699											4	-34507.15	69022.30	287.82	0.00
24	135.40		-0.5892	-0.1331						-0.0007					5	-34506.71	69023.40	288.95	0.00
22	209.20	0.0015	0.0459			-0.0462					0.0001				6	-34523.76	69059.50	325.03	0.00
6	207.70	0.0038	0.0759			-0.0461									5	-34526.28	69062.60	328.08	0.00
23	115.40	0.0073		-0.0636					0.0000						5	-34554.23	69118.50	383.98	0.00
8	116.60	0.0033		-0.0518											4	-34557.37	69122.70	388.25	0.00
25	122.40	0.0014	-0.0134								0.0001				5	-34599.98	69210.00	475.49	0.00
10	121.10	0.0036	0.0153												4	-34602.24	69212.50	478.00	0.00

Table S2.5 – Results from model selection for HF; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
46	59.18	-0.0011	0.0545	-0.1448	+	-0.0114				0.0000	0.0014					9	-24316.82	48651.70	0.00	0.33
26	59.46	-0.0012	0.0470	-0.1455	+	-0.0114				0.0000	0.0013	0.0000				10	-24315.87	48651.80	0.10	0.31
42	58.42	-0.0005	0.0592	-0.1541	+	-0.0114					0.0015	0.0000				9	-24317.08	48652.20	0.51	0.26
28	53.75	-0.0005	0.1540	-0.1525	+	-0.0089			-0.0001		0.0014	0.0000				10	-24317.00	48654.00	2.37	0.10
38	68.77	-0.0021	-0.1052	-0.0744	+	-0.0122				0.0000		0.0000				9	-24328.81	48675.60	23.98	0.00
27	20.46	0.0036	0.7951	-0.0789	+	0.0120	0.0000		-0.0005							9	-24336.58	48691.20	39.51	0.00
1	67.36	0.0004	-0.0913	-0.0780	+	-0.0120										7	-24341.26	48696.50	44.87	0.00
30	60.10	-0.0012	0.0450	-0.1449		-0.0116				0.0000	0.0013	0.0000				9	-24339.47	48697.00	45.29	0.00
48	59.79	-0.0011	0.0532	-0.1441		-0.0115				0.0000	0.0013					8	-24340.62	48697.30	45.60	0.00
44	59.07	-0.0006	0.0570	-0.1533		-0.0115					0.0015	0.0000				8	-24340.63	48697.30	45.62	0.00
34	67.44	0.0004	-0.0913	-0.0770	+	-0.0120									+	8	-24341.09	48698.20	46.52	0.00
33	67.43	0.0004	-0.0932	-0.0780	+	-0.0120								+		8	-24341.18	48698.40	46.71	0.00
32	67.35	0.0004	-0.0913	-0.0780	+	-0.0120							+			8	-24341.25	48698.50	46.86	0.00
15	57.45	0.0002	0.0852	-0.1600		-0.0113					0.0016					7	-24344.07	48702.20	50.50	0.00
21	56.85		0.0864	-0.1780		-0.0113					0.0018					6	-24350.08	48712.20	60.50	0.00
40	69.34	-0.0021	-0.1061	-0.0743		-0.0124				0.0000		0.0000				8	-24352.15	48720.30	68.66	0.00
14	69.09	-0.0020	-0.0985	-0.0727		-0.0123				0.0000						7	-24353.55	48721.10	69.45	0.00
16	68.91	-0.0009	-0.1098	-0.0788		-0.0123						0.0000				7	-24357.20	48728.40	76.74	0.00
13	32.06	0.0004	0.6320	-0.0787		0.0062			-0.0004							7	-24360.49	48735.00	83.33	0.00
12	48.12	0.0004	-0.0901	-0.2541		-0.0020		0.0001								7	-24363.02	48740.00	88.38	0.00
3	67.90	0.0004	-0.0915	-0.0778		-0.0122										6	-24364.99	48742.00	90.33	0.00
11	69.59	-0.0015	-0.0912	-0.0780		-0.0131	0.0000									7	-24364.66	48743.30	91.67	0.00
43	34.95	-0.0003	0.0951	-0.1667	+						0.0018	0.0000				8	-24368.15	48752.30	100.64	0.00
47	35.32	-0.0007	0.0936	-0.1607	+					0.0000	0.0017					8	-24368.30	48752.60	100.96	0.00
29	35.49	-0.0008	0.0875	-0.1613	+					0.0000	0.0017	0.0000				9	-24367.66	48753.30	101.67	0.00
2	65.37	0.0009		-0.0574	+	-0.0124										6	-24388.28	48788.60	136.91	0.00
39	45.46	-0.0019	-0.1071	-0.0702	+					0.0000		0.0000				8	-24388.92	48793.80	142.18	0.00
45	35.33	-0.0004	0.0933	-0.1661							0.0018	0.0000				7	-24392.69	48799.40	147.72	0.00
49	35.66	-0.0007	0.0928	-0.1601						0.0000	0.0017					7	-24393.04	48800.10	148.44	0.00
31	35.85	-0.0008	0.0859	-0.1609						0.0000	0.0017	0.0000				8	-24392.23	48800.50	148.81	0.00
19	34.34	0.0002	0.1148	-0.1712							0.0019					6	-24394.73	48801.50	149.80	0.00
24	33.80		0.1159	-0.1888							0.0021					5	-24400.47	48810.90	159.28	0.00
4	44.49	0.0004	-0.0946	-0.0737	+											6	-24399.47	48810.90	159.28	0.00
37	44.62	0.0004	-0.0945	-0.0724	+										+	7	-24399.17	48812.40	160.69	0.00
36	44.67	0.0004	-0.0982	-0.0736	+									+		7	-24399.19	48812.40	160.72	0.00
35	44.48	0.0004	-0.0945	-0.0737	+								+			7	-24399.45	48812.90	161.24	0.00
20	66.60	-0.0007		-0.0527		-0.0126				0.0000						6	-24406.81	48825.60	173.96	0.00
7	65.91	0.0009		-0.0571		-0.0125										5	-24411.94	48833.90	182.21	0.00
41	45.79	-0.0019	-0.1080	-0.0700						0.0000		0.0000				7	-24413.23	48840.50	188.82	0.00
17	45.63	-0.0018	-0.1014	-0.0686						0.0000						6	-24414.29	48840.60	188.92	0.00

18	45.48	-0.0007	-0.1115	-0.0743								0.0000				6	-24417.72	48847.40	195.78	0.00
5	44.79	0.0004	-0.0948	-0.0734												5	-24424.13	48858.30	206.59	0.00
9	46.00		-0.1360	-0.0840												4	-24440.02	48888.00	236.38	0.00
23	42.45	-0.0005		-0.0479						0.0000						5	-24469.94	48949.90	298.23	0.00
8	42.05	0.0009		-0.0519												4	-24473.88	48955.80	304.10	0.00
6	55.41	0.0017	0.1216			-0.0074										5	-24760.72	49531.40	879.78	0.00
22	55.69	0.0013	0.1165			-0.0074						0.0000				6	-24759.97	49531.90	880.28	0.00
10	41.46	0.0017	0.1120													4	-24781.37	49570.70	919.07	0.00
25	41.67	0.0013	0.1074									0.0000				5	-24780.75	49571.50	919.84	0.00

Table S2.6 – Results from model selection for Ear; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model; "+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
30	38.65	-0.0083	-0.0681	-0.0697		-0.0077				0.0000	0.0008	0.0001				9	-12332.12	24682.30	0.00	0.41
26	38.52	-0.0083	-0.0682	-0.0693	+	-0.0077				0.0000	0.0008	0.0001				10	-12331.39	24682.80	0.56	0.31
40	44.40	-0.0089	-0.1650	-0.0266		-0.0081				-0.0001		0.0001				8	-12334.13	24684.30	2.03	0.15
38	44.22	-0.0089	-0.1643	-0.0265	+	-0.0081				-0.0001		0.0001				9	-12333.37	24684.80	2.52	0.12
31	23.20	-0.0084	-0.0567	-0.0739						-0.0001	0.0010	0.0001				8	-12338.36	24692.80	10.48	0.00
29	23.15	-0.0084	-0.0569	-0.0734	+					-0.0001	0.0010	0.0001				9	-12337.58	24693.20	10.92	0.00
41	28.99	-0.0092	-0.1706	-0.0231						-0.0001		0.0001				7	-12341.15	24696.30	14.06	0.00
39	28.89	-0.0092	-0.1699	-0.0230	+					-0.0001		0.0001				8	-12340.32	24696.70	14.40	0.00
48	38.50	-0.0077	-0.0352	-0.0646		-0.0081				-0.0001	0.0008					8	-12343.60	24703.20	20.97	0.00
46	38.37	-0.0077	-0.0355	-0.0642	+	-0.0080				-0.0001	0.0008					9	-12342.83	24703.70	21.43	0.00
14	44.41	-0.0083	-0.1348	-0.0203		-0.0085				-0.0001						7	-12345.73	24705.50	23.21	0.00
28	107.40	-0.0048	-1.5010	-0.1542	+	-0.0472			0.0008		0.0022	0.0001				10	-12345.73	24711.50	29.24	0.00
49	22.36	-0.0079	-0.0226	-0.0688						-0.0001	0.0010					7	-12350.37	24714.80	32.49	0.00
47	22.31	-0.0079	-0.0229	-0.0683	+					-0.0001	0.0010					8	-12349.53	24715.10	32.83	0.00
44	33.05	-0.0048	0.0269	-0.1297		-0.0080					0.0017	0.0001				8	-12350.71	24717.50	35.19	0.00
42	32.93	-0.0048	0.0267	-0.1292	+	-0.0080					0.0017	0.0001				9	-12349.95	24717.90	35.67	0.00
17	28.33	-0.0086	-0.1399	-0.0164						-0.0001						6	-12353.33	24718.70	36.40	0.00
45	16.87	-0.0049	0.0402	-0.1349							0.0019	0.0001				7	-12357.43	24728.90	46.62	0.00
43	16.82	-0.0049	0.0398	-0.1343	+						0.0019	0.0001				8	-12356.61	24729.20	46.97	0.00
16	45.39	-0.0053	-0.1809	-0.0425		-0.0092						0.0001				7	-12360.94	24735.90	53.63	0.00
18	28.01	-0.0055	-0.1879	-0.0391								0.0001				6	-12369.76	24751.50	69.27	0.00
20	43.58	-0.0068		0.0079		-0.0102				-0.0001						6	-12385.21	24782.40	100.16	0.00
23	24.12	-0.0071		0.0138						-0.0001						5	-12396.00	24802.00	119.74	0.00
21	26.52		0.2023	-0.1859		-0.0090					0.0028					6	-12397.20	24806.40	124.16	0.00
15	26.93	0.0001	0.2036	-0.1791		-0.0092					0.0027					7	-12396.77	24807.60	125.29	0.00
24	8.20		0.2218	-0.1909							0.0030					5	-12405.57	24821.10	138.87	0.00
19	8.28	0.0001	0.2229	-0.1862							0.0029					6	-12405.36	24822.70	140.46	0.00
22	35.51	-0.0045	-0.0609			-0.0051						0.0001				6	-12409.75	24831.50	149.25	0.00
25	25.94	-0.0047	-0.0704									0.0001				5	-12412.52	24835.10	152.79	0.00
11	52.71	-0.0078	-0.1114	-0.0429		-0.0145	0.0000									7	-12421.92	24857.90	175.60	0.00
1	46.33	0.0003	-0.1102	-0.0416	+	-0.0113										7	-12422.26	24858.50	176.27	0.00
3	46.56	0.0003	-0.1109	-0.0418		-0.0113										6	-12423.40	24858.80	176.54	0.00
12	69.37	0.0003	-0.1149	0.1725		-0.0229		-0.0001								7	-12422.41	24858.80	176.58	0.00
33	46.53	0.0003	-0.1173	-0.0415	+	-0.0112								+		8	-12421.70	24859.40	177.16	0.00
27	57.88	-0.0083	-0.2134	-0.0427	+	-0.0172	0.0000		0.0001							9	-12420.76	24859.60	177.29	0.00
32	46.29	0.0004	-0.1098	-0.0415	+	-0.0113							+			8	-12421.99	24860.00	177.74	0.00
34	46.39	0.0003	-0.1101	-0.0401	+	-0.0112									+	8	-12422.07	24860.20	177.90	0.00
13	30.66	0.0003	0.2117	-0.0421		-0.0033			-0.0002							7	-12423.19	24860.40	178.13	0.00
4	24.83	0.0003	-0.1159	-0.0374	+											6	-12435.32	24882.70	200.39	0.00
36	25.25	0.0003	-0.1243	-0.0373	+									+		7	-12434.57	24883.20	200.89	0.00

5	24.95	0.0003	-0.1167	-0.0375												5	-12436.59	24883.20	200.93	0.00
37	25.04	0.0003	-0.1158	-0.0354	+										+	7	-12434.99	24884.00	201.74	0.00
35	24.77	0.0003	-0.1156	-0.0373	+							+				7	-12435.07	24884.20	201.90	0.00
9	25.86		-0.1470	-0.0450												4	-12439.78	24887.60	205.30	0.00
2	45.45	0.0009		-0.0166	+	-0.0125										6	-12448.22	24908.50	226.18	0.00
7	45.70	0.0009		-0.0166		-0.0125										5	-12449.71	24909.40	227.16	0.00
8	21.63	0.0009		-0.0104												4	-12465.73	24939.50	257.20	0.00
6	36.82	0.0010	0.0063			-0.0073										5	-12469.36	24948.70	266.46	0.00
10	23.04	0.0009	-0.0056													4	-12474.94	24957.90	275.62	0.00

Table S2.7 – Results from model selection for Mass; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
38	318.00	-0.0686	-0.4982	-0.4569	+	-0.0797				-0.0005		0.0006				9	-16311.09	32640.20	0.00	0.33
40	320.70	-0.0693	-0.5089	-0.4564		-0.0802				-0.0005		0.0006				8	-16312.27	32640.60	0.36	0.27
26	307.70	-0.0678	-0.3540	-0.5221	+	-0.0782				-0.0005	0.0012	0.0006				10	-16311.06	32642.20	1.95	0.12
30	310.40	-0.0685	-0.3647	-0.5216		-0.0787				-0.0005	0.0012	0.0006				9	-16312.24	32642.50	2.30	0.10
39	166.60	-0.0710	-0.5674	-0.4326	+					-0.0005		0.0006				8	-16314.15	32644.30	4.10	0.04
41	168.40	-0.0717	-0.5787	-0.4319						-0.0005		0.0006				7	-16315.36	32644.80	4.53	0.03
14	327.90	-0.0661	-0.2949	-0.4042		-0.0859				-0.0007						7	-16315.74	32645.50	5.27	0.02
20	330.10	-0.0634		-0.3366		-0.0913				-0.0007						6	-16316.83	32645.70	5.46	0.02
29	146.40	-0.0688	-0.1743	-0.6100	+					-0.0005	0.0033	0.0006				9	-16313.91	32645.90	5.63	0.02
31	148.10	-0.0695	-0.1840	-0.6100						-0.0005	0.0033	0.0006				8	-16315.12	32646.30	6.05	0.02
46	322.40	-0.0652	-0.2384	-0.4235	+	-0.0850				-0.0007	0.0004					9	-16314.64	32647.30	7.09	0.01
48	324.80	-0.0659	-0.2500	-0.4242		-0.0854				-0.0007	0.0004					8	-16315.73	32647.50	7.27	0.01
17	164.70	-0.0685	-0.3555	-0.3743						-0.0007						6	-16319.29	32650.60	10.36	0.00
23	154.60	-0.0653		-0.2895						-0.0007						5	-16320.90	32651.80	11.59	0.00
47	147.10	-0.0661	-0.0331	-0.5121	+					-0.0007	0.0026					8	-16318.01	32652.10	11.84	0.00
49	148.70	-0.0668	-0.0439	-0.5132						-0.0007	0.0026					7	-16319.14	32652.30	12.08	0.00
42	246.00	-0.0377	0.3019	-1.0300	+	-0.0718					0.0084	0.0012				9	-16321.56	32661.20	20.93	0.00
44	248.00	-0.0379	0.3016	-1.0390		-0.0722					0.0085	0.0012				8	-16323.09	32662.20	21.98	0.00
28	-44.71	-0.0394	6.1210	-0.9619	+	0.0797			-0.0030		0.0069	0.0012				10	-16321.26	32662.60	22.36	0.00
16	319.00	-0.0398	-0.7218	-0.6047		-0.0828						0.0013				7	-16324.77	32663.60	23.34	0.00
43	99.08	-0.0392	0.4535	-1.1000	+						0.0102	0.0012				8	-16323.94	32663.90	23.69	0.00
45	100.20	-0.0395	0.4541	-1.1090							0.0103	0.0012				7	-16325.50	32665.00	24.80	0.00
18	161.90	-0.0421	-0.7956	-0.5807								0.0013				6	-16328.04	32668.10	27.87	0.00
15	216.50	0.0133	1.6320	-1.3810		-0.0882					0.0153					7	-16350.40	32714.80	74.59	0.00
33	332.50	0.0145	0.0661	-0.6011	+	-0.1088								+		8	-16350.72	32717.50	77.24	0.00
19	34.35	0.0126	1.8510	-1.4760							0.0176					6	-16353.95	32719.90	79.69	0.00
32	343.50	0.0128	-0.1375	-0.5972	+	-0.1082							+			8	-16352.10	32720.20	80.01	0.00
2	343.60	0.0153		-0.5668	+	-0.1111										6	-16354.39	32720.80	80.57	0.00
1	342.80	0.0145	-0.1416	-0.6006	+	-0.1086										7	-16354.14	32722.30	82.08	0.00
7	347.20	0.0153		-0.5653		-0.1121										5	-16356.23	32722.50	82.23	0.00
3	346.20	0.0144	-0.1568	-0.6028		-0.1094										6	-16355.92	32723.90	83.63	0.00
34	341.20	0.0145	-0.1449	-0.6161	+	-0.1086									+	8	-16354.00	32724.10	83.82	0.00
12	175.60	0.0143	-0.1425	-2.2280		-0.0234		0.0008								7	-16355.69	32725.40	85.18	0.00
11	329.90	0.0405	-0.1471	-0.5980		-0.1012	0.0000									7	-16355.87	32725.80	85.53	0.00
13	387.50	0.0144	-0.9766	-0.6017		-0.1302			0.0004							7	-16355.92	32725.90	85.63	0.00
27	323.30	0.0411	-0.0751	-0.5958	+	-0.0988	0.0000		0.0000							9	-16354.09	32726.20	85.99	0.00
36	124.90	0.0138	-0.0071	-0.5691	+									+		7	-16356.33	32726.70	86.45	0.00
35	136.90	0.0121	-0.2098	-0.5652	+								+			7	-16357.65	32729.30	89.10	0.00
4	135.40	0.0138	-0.2142	-0.5686	+											6	-16359.73	32731.50	91.24	0.00
8	131.10	0.0151		-0.5137												4	-16362.24	32732.50	92.25	0.00

5	137.40	0.0138	-0.2302	-0.5706												5	-16361.57	32733.20	92.93	0.00
37	133.80	0.0138	-0.2175	-0.5841	+										+	7	-16359.59	32733.20	92.97	0.00
25	126.60	-0.0295	1.0200									0.0013				5	-16381.18	32772.40	132.15	0.00
22	174.30	-0.0287	1.0650			-0.0254						0.0013				6	-16380.88	32773.80	133.55	0.00
24	28.71		1.4000	-2.2180							0.0253					5	-16389.73	32789.50	149.24	0.00
21	110.20		1.2910	-2.1930		-0.0395					0.0245					6	-16389.01	32790.10	149.81	0.00
9	182.30		-1.7790	-0.9640												4	-16405.74	32819.50	179.25	0.00
6	201.70	0.0252	1.6210			-0.0520										5	-16410.62	32831.30	191.02	0.00
10	103.40	0.0246	1.5390													4	-16411.89	32831.80	191.56	0.00

Table S2.8 – Results from model selection for TL/TBL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
26	0.67	0.0000	-0.0015	0.0006	+	-0.0001				0.0000	0.0000	0.0000				10	17903.85	-35787.70	0.00	0.74
46	0.67	0.0000	-0.0014	0.0006	+	-0.0001				0.0000	0.0000					9	17901.63	-35785.20	2.44	0.22
30	0.67	0.0000	-0.0015	0.0006		-0.0001				0.0000	0.0000	0.0000				9	17899.57	-35781.10	6.57	0.03
48	0.67	0.0000	-0.0014	0.0006		-0.0001				0.0000	0.0000					8	17897.44	-35778.90	8.82	0.01
42	0.70	0.0000	-0.0018	0.0008	+	-0.0001					0.0000	0.0000				9	17890.68	-35763.30	24.34	0.00
28	0.66	0.0000	-0.0010	0.0008	+	-0.0001			0.0000		0.0000	0.0000				10	17890.76	-35761.50	26.19	0.00
38	0.59	0.0000	-0.0001	0.0000	+	-0.0001				0.0000		0.0000				9	17888.57	-35759.10	28.57	0.00
15	0.70	0.0000	-0.0018	0.0008		-0.0001					0.0000					7	17886.28	-35758.50	29.14	0.00
21	0.70		-0.0018	0.0008		-0.0001					0.0000					6	17885.04	-35758.10	29.61	0.00
44	0.70	0.0000	-0.0018	0.0008		-0.0001					0.0000	0.0000				8	17886.42	-35756.80	30.86	0.00
20	0.59	0.0000		0.0000		-0.0001				0.0000						6	17882.51	-35753.00	34.66	0.00
40	0.59	0.0000	-0.0001	0.0000		-0.0001				0.0000		0.0000				8	17884.34	-35752.70	35.01	0.00
14	0.59	0.0000	-0.0001	0.0000		-0.0001				0.0000						7	17882.74	-35751.50	36.21	0.00
27	0.83	0.0000	-0.0047	0.0001	+	-0.0002	0.0000		0.0000							9	17863.25	-35708.50	79.21	0.00
33	0.61	0.0000	-0.0002	0.0001	+	-0.0001								+		8	17861.68	-35707.30	80.35	0.00
1	0.61	0.0000	-0.0001	0.0001	+	-0.0001										7	17860.53	-35707.10	80.63	0.00
32	0.61	0.0000	-0.0001	0.0001	+	-0.0001							+			8	17861.33	-35706.60	81.03	0.00
2	0.60	0.0000		0.0001	+	-0.0001										6	17858.86	-35705.70	81.98	0.00
34	0.61	0.0000	-0.0001	0.0001	+	-0.0001									+	8	17860.79	-35705.60	82.12	0.00
13	0.83	0.0000	-0.0046	0.0001		-0.0002			0.0000							7	17859.15	-35704.30	83.39	0.00
16	0.60	0.0000	0.0000	0.0001		-0.0001						0.0000				7	17859.05	-35704.10	83.60	0.00
3	0.60	0.0000	-0.0001	0.0001		-0.0001										6	17856.26	-35700.50	87.16	0.00
11	0.58	0.0000	-0.0001	0.0001		-0.0001	0.0000									7	17857.08	-35700.10	87.54	0.00
12	0.68	0.0000	-0.0001	0.0008		-0.0001		0.0000								7	17856.77	-35699.50	88.15	0.00
7	0.60	0.0000		0.0001		-0.0001										5	17854.59	-35699.20	88.50	0.00
22	0.61	0.0000	-0.0002			-0.0001						0.0000				6	17854.04	-35696.10	91.61	0.00
6	0.61	0.0000	-0.0003			-0.0001										5	17852.00	-35694.00	93.68	0.00
29	0.46	0.0000	-0.0011	0.0005	+					0.0000	0.0000	0.0000				9	17832.28	-35646.50	141.15	0.00
47	0.45	0.0000	-0.0010	0.0005	+					0.0000	0.0000					8	17830.49	-35645.00	142.71	0.00
31	0.45	0.0000	-0.0011	0.0005						0.0000	0.0000	0.0000				8	17828.50	-35641.00	146.69	0.00
49	0.45	0.0000	-0.0010	0.0005						0.0000	0.0000					7	17826.80	-35639.60	148.10	0.00
39	0.41	0.0000	-0.0001	0.0000	+					0.0000		0.0000				8	17824.30	-35632.60	155.10	0.00
23	0.40	0.0000		0.0001						0.0000						5	17818.69	-35627.40	160.31	0.00
41	0.41	0.0000	-0.0001	0.0000						0.0000		0.0000				7	17820.55	-35627.10	160.60	0.00
17	0.40	0.0000	-0.0001	0.0000						0.0000						6	17819.18	-35626.30	161.34	0.00
43	0.48	0.0000	-0.0015	0.0007	+						0.0000	0.0000				8	17815.67	-35615.30	172.35	0.00
19	0.48	0.0000	-0.0016	0.0007							0.0000					6	17811.28	-35610.60	177.12	0.00
24	0.48		-0.0016	0.0007							0.0000					5	17810.19	-35610.40	177.31	0.00
45	0.48	0.0000	-0.0015	0.0007							0.0000	0.0000				7	17811.94	-35609.90	177.82	0.00

36	0.42	0.0000	-0.0002	0.0001	+									+		7	17796.32	-35578.60	209.05	0.00
4	0.42	0.0000	-0.0002	0.0001	+											6	17794.55	-35577.10	210.59	0.00
35	0.42	0.0000	-0.0002	0.0001	+								+			7	17795.44	-35576.90	210.82	0.00
18	0.41	0.0000	-0.0001	0.0001								0.0000				6	17794.17	-35576.30	211.36	0.00
37	0.42	0.0000	-0.0002	0.0001	+										+	7	17794.96	-35575.90	211.78	0.00
9	0.41		-0.0002	0.0001												4	17790.72	-35573.40	214.25	0.00
5	0.42	0.0000	-0.0002	0.0001												5	17790.75	-35571.50	216.19	0.00
8	0.41	0.0000		0.0001												4	17788.35	-35568.70	218.98	0.00
25	0.42	0.0000	-0.0004									0.0000				5	17782.62	-35555.20	232.45	0.00
10	0.42	0.0000	-0.0004													4	17780.42	-35552.80	234.85	0.00

Table S2.9 – Results from model selection for HF/TBL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; yer = year; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight = model weight, the relative likelihood of a model;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	sex	yer	Elv:yer	Lng:yer	Ltt:yer	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:sex	Ltt:sex	Lng:sex	df	logLik	AICc	delta	weight
m42	0.15	0.0000	0.0005	-0.0002	+	0.0000					0.0000	0.0000				9	23515.81	-47013.60	0.00	0.37
m28	0.20	0.0000	-0.0005	-0.0002	+	0.0000		0.0000			0.0000	0.0000				10	23516.39	-47012.80	0.84	0.24
m26	0.15	0.0000	0.0005	-0.0002	+	0.0000				0.0000	0.0000	0.0000				10	23516.04	-47012.10	1.53	0.17
m44	0.15	0.0000	0.0005	-0.0002		0.0000					0.0000	0.0000				8	23513.44	-47010.90	2.74	0.10
m30	0.15	0.0000	0.0005	-0.0002		0.0000				0.0000	0.0000	0.0000				9	23513.67	-47009.30	4.26	0.04
m43	0.13	0.0000	0.0005	-0.0002	+						0.0000	0.0000				8	23512.07	-47008.10	5.47	0.02
m29	0.12	0.0000	0.0006	-0.0002	+					0.0000	0.0000	0.0000				9	23512.43	-47006.80	6.76	0.01
m46	0.15	0.0000	0.0004	-0.0002	+	0.0000				0.0000	0.0000					9	23512.35	-47006.70	6.92	0.01
m27	0.05	0.0000	0.0020	0.0000	+	0.0001	0.0000		0.0000							9	23511.74	-47005.50	8.13	0.01
m45	0.13	0.0000	0.0005	-0.0002							0.0000	0.0000				7	23509.62	-47005.20	8.37	0.01
m48	0.15	0.0000	0.0004	-0.0002		0.0000				0.0000	0.0000					8	23510.09	-47004.20	9.44	0.00
m31	0.12	0.0000	0.0006	-0.0002						0.0000	0.0000	0.0000				8	23509.98	-47003.90	9.65	0.00
m11	0.15	0.0000	0.0000	0.0000		0.0000	0.0000									7	23508.03	-47002.00	11.54	0.00
m38	0.17	0.0000	0.0001	0.0000	+	0.0000					0.0000	0.0000				9	23509.82	-47001.60	11.97	0.00
m47	0.13	0.0000	0.0005	-0.0002	+					0.0000	0.0000					8	23508.53	-47001.10	12.54	0.00
m15	0.16	0.0000	0.0003	-0.0002		0.0000				0.0000						7	23507.41	-47000.80	12.79	0.00
m16	0.17	0.0000	0.0001	0.0000		0.0000						0.0000				7	23507.39	-47000.80	12.83	0.00
m40	0.17	0.0000	0.0001	0.0000		0.0000				0.0000		0.0000				8	23507.47	-46998.90	14.66	0.00
m2	0.18	0.0000		0.0000	+	0.0000										6	23505.46	-46998.90	14.69	0.00
m49	0.13	0.0000	0.0005	-0.0002						0.0000	0.0000					7	23506.20	-46998.40	15.21	0.00
m1	0.18	0.0000	0.0000	0.0000	+	0.0000										7	23505.95	-46997.90	15.71	0.00
m33	0.18	0.0000	0.0000	0.0000	+	0.0000								+		8	23506.71	-46997.40	16.19	0.00
m7	0.18	0.0000		0.0000		0.0000										5	23503.23	-46996.50	17.14	0.00
m34	0.18	0.0000	0.0000	0.0000	+	0.0000									+	8	23506.14	-46996.30	17.32	0.00
m32	0.18	0.0000	0.0000	0.0000	+	0.0000							+			8	23506.13	-46996.20	17.36	0.00
m3	0.18	0.0000	0.0000	0.0000		0.0000										6	23503.72	-46995.40	18.16	0.00
m20	0.18	0.0000		-0.0001		0.0000					0.0000					6	23503.67	-46995.30	18.27	0.00
m14	0.18	0.0000	0.0000	0.0000		0.0000					0.0000					7	23504.33	-46994.60	18.95	0.00
m12	0.21	0.0000	0.0000	0.0003		0.0000	-	0.0000								7	23504.15	-46994.30	19.31	0.00
m19	0.13	0.0000	0.0004	-0.0002							0.0000					6	23502.94	-46993.90	19.71	0.00
m13	0.19	0.0000	-0.0003	0.0000		0.0000				0.0000						7	23503.80	-46993.60	20.01	0.00
m39	0.15	0.0000	0.0001	0.0000	+					0.0000		0.0000				8	23504.74	-46993.50	20.13	0.00
m18	0.15	0.0000	0.0001	0.0000								0.0000				6	23502.23	-46992.50	21.13	0.00
m22	0.17	0.0000	0.0002			0.0000						0.0000				6	23501.47	-46990.90	22.67	0.00
m41	0.15	0.0000	0.0001	0.0000						0.0000		0.0000				7	23502.30	-46990.60	23.01	0.00
m4	0.15	0.0000	0.0000	0.0000	+											6	23500.57	-46989.10	24.46	0.00
m36	0.15	0.0000	0.0000	0.0000	+									+		7	23501.47	-46988.90	24.66	0.00
m21	0.16		0.0003	-0.0001		0.0000					0.0000					6	23500.29	-46988.60	25.01	0.00
m8	0.15	0.0000		0.0000												4	23497.85	-46987.70	25.89	0.00
m37	0.15	0.0000	0.0000	0.0000	+										+	7	23500.80	-46987.60	26.00	0.00

m35	0.15	0.0000	0.0000	0.0000	+								+		7	23500.77	-46987.50	26.07	0.00
m23	0.15	0.0000		0.0000						0.0000					5	23498.41	-46986.80	26.78	0.00
m5	0.15	0.0000	0.0000	0.0000											5	23498.26	-46986.50	27.09	0.00
m17	0.15	0.0000	0.0000	0.0000						0.0000					6	23498.99	-46986.00	27.62	0.00
m25	0.15	0.0000	0.0002									0.0000			5	23497.73	-46985.40	28.15	0.00
m6	0.17	0.0000	0.0001			0.0000									5	23496.68	-46983.30	30.25	0.00
m24	0.14		0.0004	-0.0001							0.0000				5	23495.90	-46981.80	31.79	0.00
m9	0.15		0.0001	0.0000											4	23493.55	-46979.10	34.49	0.00
m10	0.15	0.0000	0.0001												4	23492.77	-46977.50	36.05	0.00

CHAPTER 3

Range-wide variation in skull morphometry in the American red squirrel, *Tamiasciurus hudsonicus*

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ABSTRACT

Environmental factors, both abiotic and biotic, provide important selective pressures on species and can shape both their morphology and genetic makeup. For many species large scale factors such as temperature and elevation can both determine the range limits and physiological limits attained by adaptation. In mammals, adaptation to low temperature is often linked with adaptation to high elevation since temperature goes down with increased elevation. A number of ecogeographical rules have been established to describe patterns of morphological variation observed in response to temperature changes, three of which are applicable to skull morphology. In response to cold, Bergmann's rule posits increased size and Allen's rule posits reduced protuberances/appendages in order to reduce heat loss (minimizing the surface area to volume ratio). Dehnel's phenomenon is a reduction in size, especially skull depth, in winter compared to other seasons, thereby reducing absolute energy expenditure.

In this study we examined skull features of the American red squirrel, *Tamiasciurus hudsonicus*, a small arboreal mammal found throughout most of Alaska and Canada and down the Rocky Mountains and the Appalachians in the US. We used multimodel inference to explore how elevation, latitude, longitude, season and sex impact a suite of skull variables (length, width, depth and volume measurements, and including ratios).

Our results provide support for Bergmann's rule, with the skull tending to be larger both at higher elevation and latitude indicating adaptive change in relation to temperature. Seasonal changes in skull morphology do not relate to Dehnel's phenomenon in a straightforward way. Allen's rule was tested using nose length (estimated by measuring the nasal bones). We did find a decline in nose length with latitude, but not with elevation; different functional adaptations may underlie these two contrasting trends.

INTRODUCTION

Wild mammals are found over an extraordinary range of environments on the planet (Macdonald 2009). While some are only found in a limited area or active only for limited times others are very widespread and are active for a large part of the day or year. The activity patterns are clearly going to impact the exposure of individuals to the environment where they live, with some mammals substantially limiting that exposure (e.g. daily and seasonal dormancy in the edible dormouse: Wilz & Heldmaier 2000). Overall, the current distribution of a species can be explained by a combination of ecological factors (ability to live in particular abiotic and biotic environments), which we are focusing on here, and historical factors (relating to their origin, their dispersion from there and influences of humans restricting where the species can live) (Cox, Moore & Ladle 2016). The manner in which species are adapted to that environment in which they live will be reflected in morphology, and some of the most exquisite adaptations reflect that, responding to biotic resources, competition, predation, abiotic conditions etc. More wide ranging species generally show more striking morphological variation since they are exposed to a greater range of environmental conditions that can shape their morphology, and this can be seen very readily in mammals (e.g. vervet monkey: Elton, Dunn & Cardini 2010). In some cases researchers have felt it is possible to generalize about this variation in morphology. Various so-

called ecogeographical rules have been proposed to explain patterns that have been observed in mammals, and other groups, as a reaction to the environment they live in (Cox, Moore & Ladle 2016).

Here we focus on thermal adaptation in a biogeographical context. The ecogeographical rules relating to this provide a backdrop to that consideration. Bergmann's rule is important in this respect. First proclaimed by Carl Bergmann in 1847, it is one of the oldest and most widely cited ecogeographical rules, which was originally based on work on endotherms and states that within a large geographical range larger individuals are found in colder environments, i.e. at higher latitudes and elevation, and smaller individuals in warmer areas (Clauss et al 2013). The original, and widely accepted, explanation for this is that larger individuals have a smaller surface area to volume ratio and will therefore lose less energy through radiation in colder environments while smaller animals will be able to increase heat loss in warmer climates (Clauss et al 2013). For an endotherm, substantial resources may be needed to maintain a core body temperature under cold conditions (Ruben 1995); therefore Bergmann's rule makes intuitive sense as a means to mitigate that cost. In a mammalian context, larger species may not only be at an advantage in cold conditions because of minimizing their surface area to volume ratio, they may also be better able to increase the density and/or length of hair to prevent heat loss than smaller species; however, empirical data are equivocal on this point (Freckleton, Harvey & Pagel 2003; Wasserman & Nash 1979).

There has been much consideration of the validity of Bergmann's rule in mammals (e.g. Ashton, Tracy & de Queiroz 2000; Meiri & Dayan 2003). In addition to the influence of temperature, it has been suggested that there may be other selective pressures influencing geographical variation in body size of endotherms such as food supply (McNab 1971; Ralls &

Harvey 1985). It has even been hypothesized that if food supplies decline with lower temperature, as is often the case, individuals may be expected to show decreased size since larger bodies are more energetically costly and will require more resources (McNab 1971). Additionally, when resources are scarce, larger individuals may be favored because they need to forage over a larger area or since they might better be able to withstand periods of starvation. For these reasons some authors have suggested that biotic factors may be more important than abiotic when it comes to body size determination (Freckleton, Harvey & Pagel 2003). These alternative explanations have been applied to mammals that show the converse trends to those expected under Bergmann's rule, e.g. in tuco-tucos (Medina, Martí & Bidau 2007).

Building on the same original explanation as Bergmann's rule, i.e. reduction of heat loss in colder climates, Allen's rule (dating to 1877) predicts that certain external body parts will vary more than body size in endotherms with the peripheral parts, such as nose, ears and feet, decreasing in size under low temperature towards the poles and at higher elevation. The rule has been applied to certain mammals such as jack rabbits (Griffing 1974) and hominins (e.g. Tilkins et al 1987). Again, smaller protuberances/appendages would be expected to minimize heat loss under cold conditions and larger elements maximize heat loss under hot conditions, because of the large surface area to volume ratio associated with such structures.

A final 'rule' relating to temperature adaptation is Dehnel's phenomenon, first described in 1949. This relates to changes in body size and other features such as skull depth during the annual cycle, with individuals decreasing in size in winter and increasing again in size come spring (Pucek 1970). While Bergmann's rule and Allen's rule have been found to hold true for a variety of taxa, both intra- and interspecifically, Dehnel's phenomenon has to date only been described for mammalian species in the shrew genus *Sorex* and in some within the rodent

subfamily Arvicolinae (Iverson & Turner 1974; Merritt & Zegers 1991; Churchfield, Rychlik & Taylor 2012). The phenomenon is associated with lowered energy expenditure through reduced body mass, not only by loss of fat reserves but reduction in the size of the skeleton, including the skull, and the brain. Dehnel's phenomenon is consistent with arguments from McNab (1971) and others against an energetic basis for Bergmann's rule, given that the absolute energetic requirements for a larger individual are greater than the absolute energetic requirements for a smaller individual. Thus, Dehnel's phenomenon aligns with the converse of Bergmann's rule, and indeed in shrews of the genus *Sorex*, their geographic variation in body size follows a converse Bergmann trend (Ochocińska & Taylor 2003). Thus, for these shrews, it appears that absolute energetic requirements may be the driving force behind body size evolution.

The three rules, ecogeographical or seasonal, that we have described are far from universal in mammals. However, they have a clear mechanistic basis, and can act as null models when considering geographical and seasonal variation in morphology in mammals. Here we describe morphological variation in a very widespread species which is active all the year round and therefore shows high exposure to temperature extremes. We examine specimens throughout that distribution thereby not restricted to simple transects along particular geographical axes (e.g. latitude) allowing a more multivariate approach on the relationship of morphology to geography. That geography can be interpreted in terms of thermal conditions, as can the season in which each individual is caught. We focus on geography rather than direct temperature measurements to test for these rules based on their original assumptions. Our methodology allows a richer approach to the ecogeographical rules than is normally possible.

Our study species is the American red squirrel (*Tamiasciurus hudsonicus*). This is a small mammal (110-255 g; Reid 2006; Bowers, Bowers & Kaufman 2007) found in coniferous forests

throughout northern North American and south along the Appalachian Mountains in the east and the Rocky Mountains in the west (figure 3.1; Steele 1998). *T.hudsonicus* is active all year round in areas spanning wide temperature ranges, from an average coldest temperature during January more extreme than -30C (with the average high around -20C at the same time) to areas with July average temperatures higher than 30C (with lowest temperature close to 20C). Within a single location the species may face either extreme annual cycles with low average temperatures in January below -20C and high average temperatures over 25C, or more moderate cycles with July annual temperature around 30C and average low January temperature at around -3C (all temperature data from http://www.usclimatedata.com). Given that the species is most active during dawn and dusk some of the extreme temperatures may be expected to be avoided through behavioral modification, however they are reliant on diurnal vision and are active at all times of day at all times of the year thereby limiting the extent of behavioral avoidance possible. Therefore, they are still bound to be seriously impacted by an extraordinary range of temperatures.

Over the last 150 years museum collections have become a crucial repository for biological specimens (Suarez & Tsutsui 2004). In the case of mammals the standard items preserved from collection expeditions are whole study skins, and even more commonly skulls. Species such as *T.hudsonicus* are well represented in major museums in the USA with collections spanning wide elevational and latitudinal range, providing large numbers of skulls for analysis. Mammalian skulls reflect body size (relating to Bergmann's rule), and the nose can be measured (Allen's rule) and skull variation with season can be examined (Dehnel's phenomenon). For these reasons it is possible through skull measurements, and with reference to

the ecogeographical rules relating to thermal conditions, to answer the question 'to what extent is the morphology of *T.hudsonicus* shaped by temperature?'

MATERIALS AND METHODS

Specimens

Skulls were accessed at the Cornell University Museum of Vertebrates (CUMV), the American Museum of Natural History (AMNH), the Field Museum of Natural History (FMNH) and the National Museum of Natural History (NMNH) and were derived from individuals that had been collected in the years between 1876 and 2004 throughout a large part of the species distribution (figure 3.1). A small minority of the specimens had coordinates already associated with the collection site. Coordinates for the remaining specimens were found using Google Earth (version 7.1.5.1557 using the WGS84 reference system, http://earth.google.com) and using information from primary literature and historical documents on a case by case basis. Specimens that could not be accurately georeferenced were dropped from analysis.
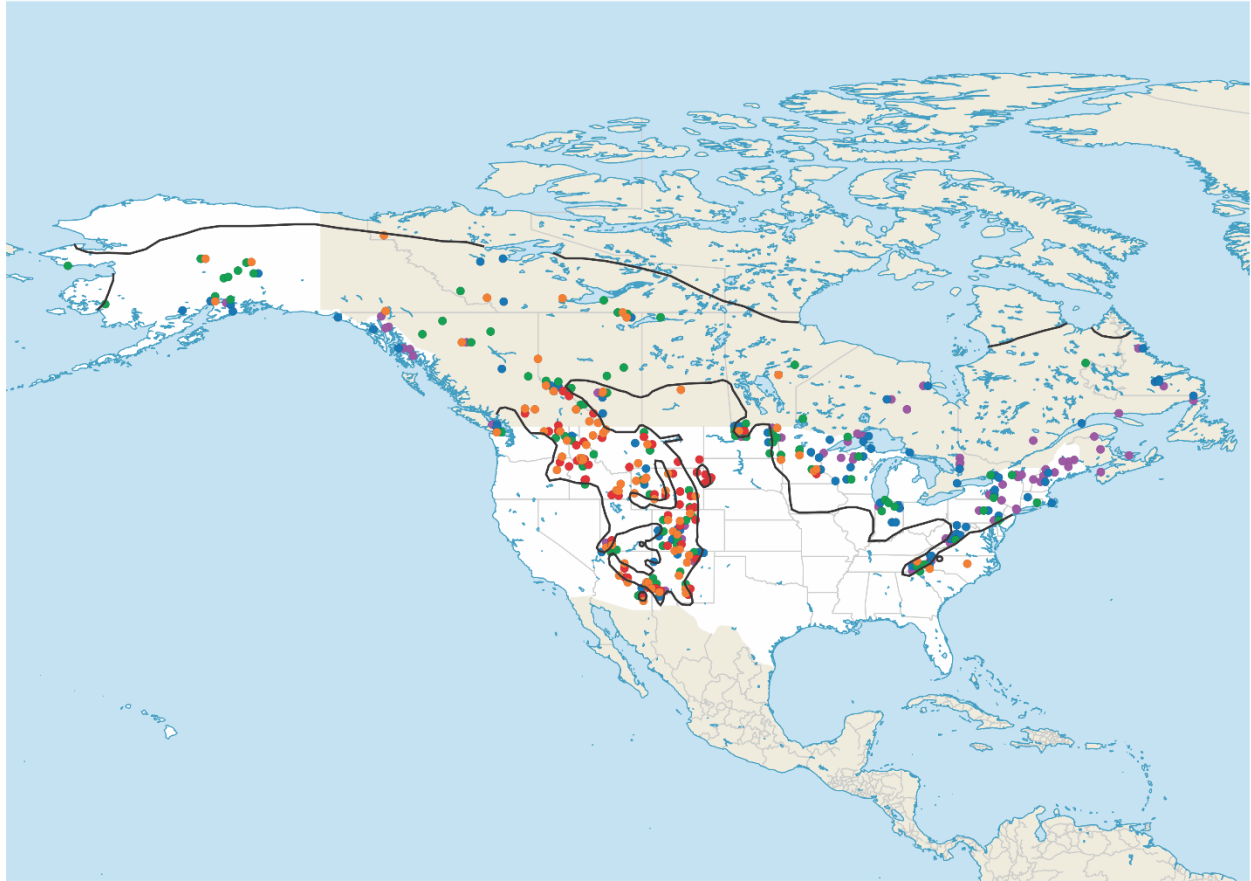
Figure 3.1 – Distribution of the American red squirrels, collection sites for specimens used in this study and size data. The distribution is indicated by a solid line and follows Steele (1998). Colors indicate categories for TSL (total skull length), with equal representation of each category. Red = 45.8-49.6mm, orange = 44.9-45.8mm, green = 43.9-44.9mm, blue = 42.8-43.9mm, purple = 39.7-42.8mm. In some cases sample points were moved slightly to allow all points to be visible.

All specimens included in the analysis were adults. For skulls where the age of the specimen had not been determined by the collector, approximate age was determined either by tooth wear or inspection of the accompanying skin (coloration of scrotum and presence of mammae; Flyger & Gates 1982). Data on weight and total body length was not used as an indicator of age due to difference in size of described subspecies (Steele 1998). We used a simplified estimation of age categories based on Layne (1954), Nellis (1969) and Lindsay (1987) and classified adults as individuals where complete permanent dentition was present and wear

was found on all molars. The age of what was classified as adults was most likely no less than five months (Nellis 1969).

Skull measurements

Skull measurements were recorded using digital calipers to the nearest 0.01mm. We estimated eight skull measures (figure 3.2): total skull length (TSL; the distance from the anterior end of the nasal bones to the surface of the occipital condyles), condylobasal length (CBL; the distance from the prosthion to the posterior surface of the occipital condyles), least interorbital breadth or eye width (EW), maximum zygomatic width (MZW), length of diastema (LD), length of posterior occipital suture (POS), nasal bone length (NB) and distance from occipital suture
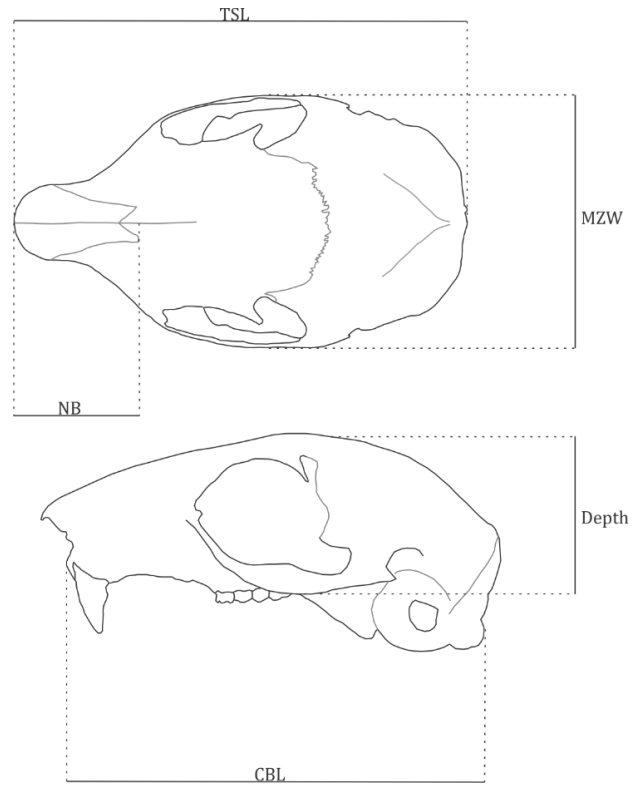

Figure 3.2 – Skull measures used in the study: TSL = total skull length; NB = nasal bone length; MZW = maximum zygomatic width; CBL = condylobasal length; Depth.

to posterior vomer (Depth). These measurements were chosen based on previous work (Findley 1961; Lindsay 1987). Three measurements were dropped from the analysis due to lack of repeatability of measurements (LD and POS) and lack of homology (EW). Additionally, we calculated the ratio of nasal bones to total skull length, NB/TSL (TSL was used as opposed to CBL since CBL does not include the total length of the nasal bones), and the ratio of the maximum zygomatic width to condylobasal length (MZW/CBL).

The volume of the brain cavity was measured by pouring 1mm glass beads into the skull, gently shaking the skull until all beads were settled and level with the foramen magnum and then pouring the beads into a graduated cylinder and measuring to the nearest 0.1ml (Volume). A simpler measure of endocranial volume was made by multiplying three other measurements: Depth, MZW, CBL (Cranial box) (following Logan & Clutton-Brock 2012). In cases where tissue remains were found within the brain cavity, Volume was not measured. When skulls were partially damaged measurements were dropped accordingly.

Analysis

We used multimodel inference to explore how skull variables are impacted by geographical factors (latitude, longitude and elevation), season and sex (Burnham & Anderson 2002). We did not include year as a factor in the analysis because of biased sampling by year in the data set. The collinearity of these factors was determined using a variance inflation factor test in R using the car package (R version 1.15.6, R Core Team 2016; Fox et al 2016) and determined to be within an acceptable range to allow the factors to be used for further analysis. We evaluated a total of 49 models (table S3.1) that were determined most suitable based on the known biology of the species. We used the second-order Akaike information criterion (AIC_c) to compare models – the best-fitting model in the set of candidate models is indicated by the lowest AICc value, and every other model i is compared to the best model using the AICc differences (Δ_i). By convention, we consider models with $\Delta AICc < 2$ (Burnham & Anderson 2002). We used the MuMIn package in R (version 1.15.6, R Core Team 2016; Bartoń 2015) to select the top models for each skull variable (we excluded TSL from the analysis since TSL and CBL were extremely similar measurements and highly correlated).

RESULTS

The model most often found to well describe the variation in the skull measurements included four out of the five main factors, i.e. elevation, latitude, longitude and sex, and the interactions elevation:longitude, elevation:latitude and longitude:latitude (table 3.1). This model was well supported for six out of the eight skull variables and the best model for four of those (where the best model is defined as $\Delta_i = 0$ and well supported models defined as $\Delta_i < 2$). Among these eight skull variables, the factors found in the well supported models were as follows: latitude, elevation and longitude in all models, sex in nine and season in six. The interaction between elevation and longitude was found in five models, between elevation and latitude in five and between latitude and longitude in six. Interaction between season and longitude was only found for MZW/CBL and interactions involving sex were only found for Depth (sex:elevation and sex:longitude).

Table 3.1 – Well supported models for all skull variables in the study (as defined by Δi <2) based on the following factors: elevation (Elev), latitude (Lat), longitude (Long), sex and season, with the best models (Δi = 0) shown in bold

Model	Variable	AIC$_c$*	Δ$_i$*	W$_i$*
Elev + Lat + Sex + Long + Elev:Long + Elev:Lat + Long:Lat	**MZW**	**1802.5**	**0**	**0.275**
	CBL	**2238.9**	**0**	**0.625**
	NB/TSL	**1773.7**	**0**	**0.408**
	Cranial box	**-3529.0**	**0**	**0.369**
	NB	1622	0.16	0.3
	Volume	214.0	0.32	0.325
Elev + Lat + Season + Sex + Long + Elev:Long + Elev:Lat + Long:Lat	Cranial box	11121.3	0.2	0.335
	MZW	1803.9	1.31	0.143
	NB	1623.4	1.39	0.162
	NB/TSL	-3527.2	1.81	0.165
Elev + Lat + Sex + Long + Elev:Long + Long:Lat	**Volume**	**213.7**	**0**	**0.381**
	MZW	1802.6	0.09	0.263
Elev + Lat + Sex + Long + Lat:Long + Elev:Lat	**NB**	**1622**	**0**	**0.325**
	CBL	2240.9	1.98	0.232
Elev + Lat + Season + Sex + Long + Elev:Long + Long:Lat	MZW	1803.7	1.18	0.153
	Volume	215.5	1.84	0.152
Elev + Lat + Season + Sex + Long + Lat:Long + Elev:Lat	NB	1623.1	1.14	0.184
Elev + Season + Lat + Long + Season:Long	**MZW/CBL**	**-3467.4**	**0**	**0.316**
Elev + Season + Sex + Lat + Long + Sex:Elev	**Depth**	**944.2**	**0**	**0.517**
Elev + Season + Sex + Lat + Long + Sex:Long	Depth	945.9	1.68	0.223
Elev + Lat + Sex + Long + Elev:Long + Elev:Lat	NB/TSL	-3527.2	1.82	0.164

* AIC$_c$ = second-order Akaike information criterion; Δ$_i$ difference between a model and the highest scoring mode; W$_i$ = model weight, the relative likelihood of a model.

Considering each of the eight skull variables in turn, tables S3.2-S3.9 provide details of all the models compared variable-by-variable and their ranking according to Δ_i score. For CBL, MZW, NB, NB/TSL, Volume and Cranial box it is evident that elevation, latitude and longitude and interactions between them are important factors as is sex. For Depth, the factors elevation, latitude, longitude, season and sex are all important, but only interaction terms associated with sex are important. The results with MZW/CBL are completely different from all the others. Only one model is well supported and the relative likelihood of that model is only 0.316. In comparison the combined likelihood of the well supported models for other skull variables ranged from 0.737 (NB/TSL) to 0.971 (NB).

It is also informative to consider each factor in turn, adopting a univariate approach. Figures 3.3 – 3.5 relate to the geographical factors, elevation, latitude and longitude. If Allen's rule was supported we would expect to see shorter noses at high elevation and latitude. If Bergmann's rule was supported we would expect to see all variables to have greater values at high elevation and latitude. Elevation shows a positive relation with all skull variables other than Depth and MZW/CBL (figure 3.3), the relationships between latitude and most variables is weak (figure 3.4) and there is a negative relationship between longitude and most variables other than MZW/CBL (figure 3.5). The relationship between elevation and TSL is well illustrated by plotting TSL categories on a map of North America (figure 3.1). The largest values tend to be found in the high elevation areas in the Rocky Mountains.

With regards geographical interactions, the largest specimens were most often found at high elevation (i.e. in the southwest) and at low elevation far north. The smaller specimens were more often found at low elevation, in the east and along the west coast. These relationships are evident in figure 3.1.
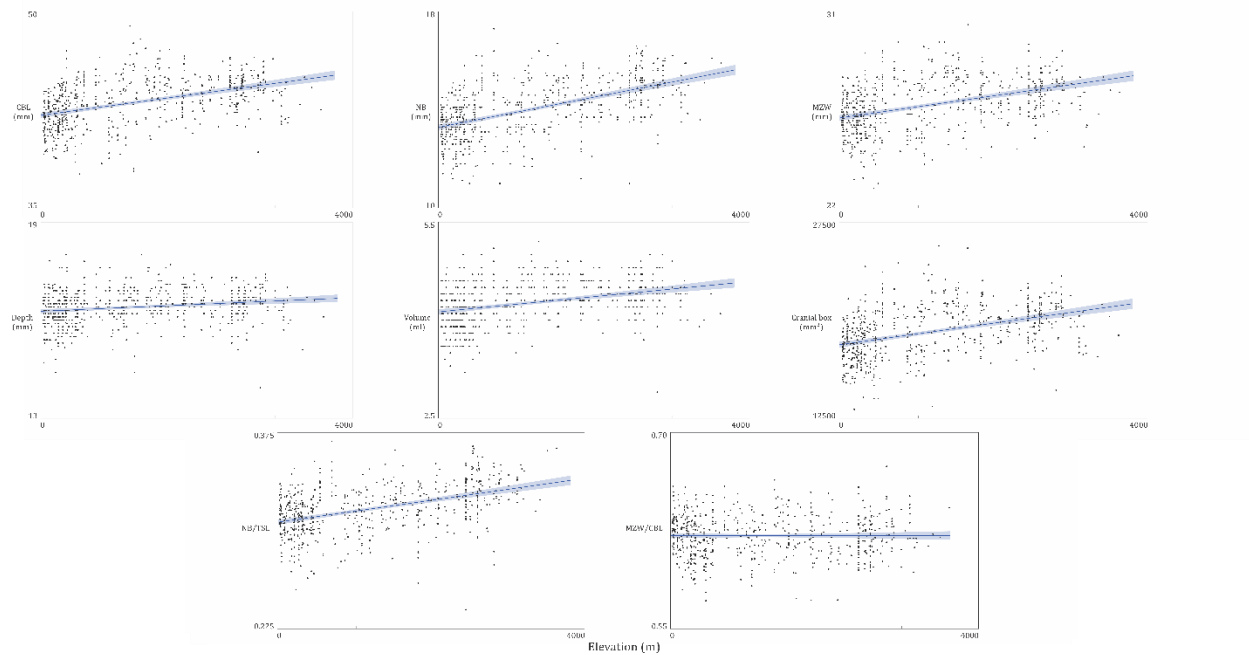
Figure 3.3 – Bivariate plot between elevation and skull variables, with a linear regression and 95% confidence intervals
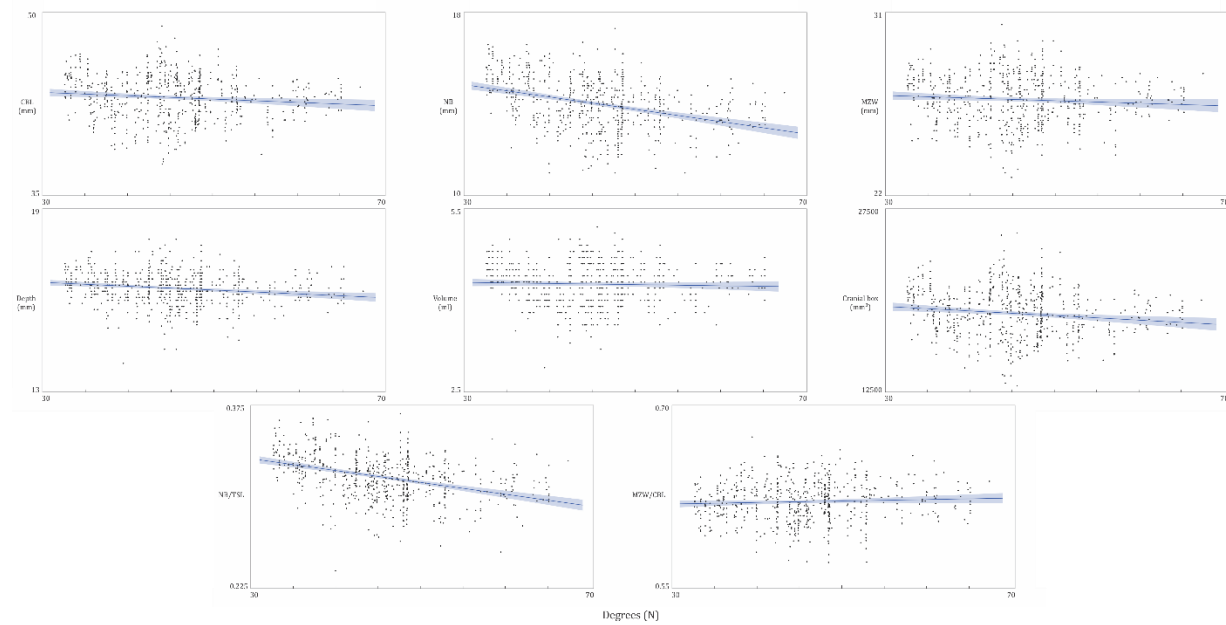
Figure 3.4 – Bivariate plot between latitude and skull variables, with a linear regression and 95% confidence intervals
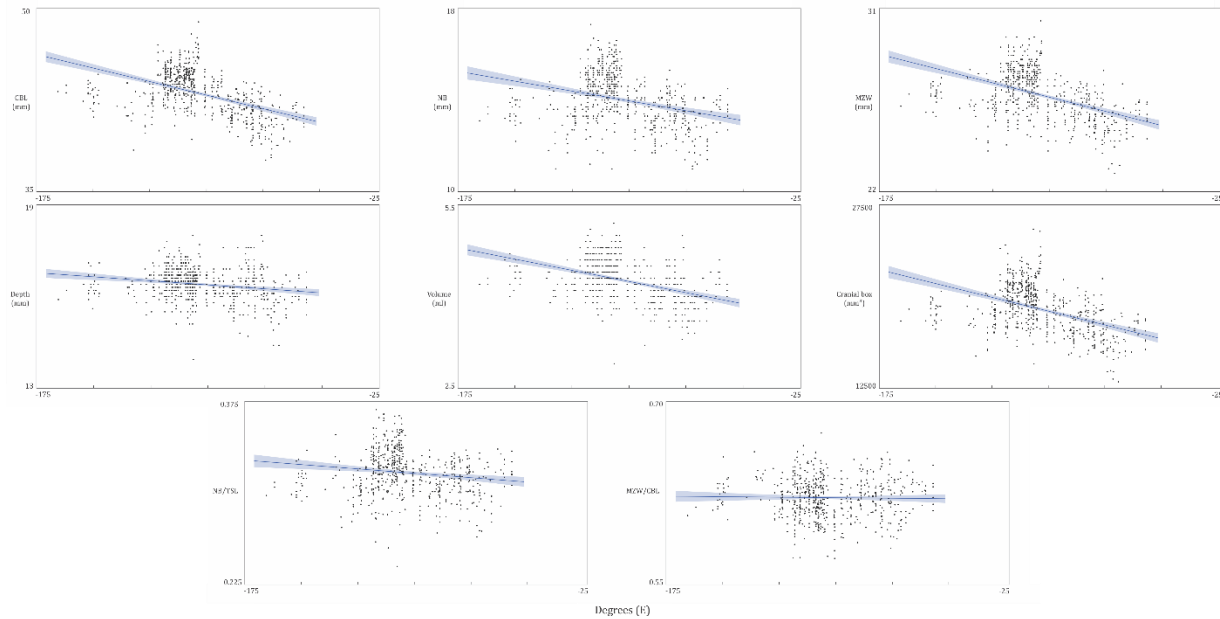
Figure 3.5 – Bivariate plot between longitude and skull variables, with a linear regression and 95% confidence intervals

With regards to the sexes, males were larger than females for all variables other than

NB/TSL and MZW/CBL (table 3.2). Geographic location did not impact this.

Table 3.2 – Means and standard deviations for each skull variable for males and females

	Males	**Females**
CBL (mm)	43.26 ± 1.90	42.84 ± 1.73
NB (mm)	14.10 ± 1.15	13.94 ± 1.09
MZW (mm)	26.86 ± 1.25	26.60 ± 1.18
Depth (mm)	16.46 ± 0.56	16.34 ± 0.51
Volume (ml)	4.32 ± 0.33	4.22 ± 0.34
NB/TSL	0.32 ± 0.02	0.32 ± 0.02
MZW/CBL	0.62 ± 0.02	0.62 ± 0.02
Cranial box (mm^3)	19221.65 ± 2085.06	18680.40 ± 2085.29

The effect of season was not constant between variables with most showing individuals

being largest in the spring and smallest in the winter (table 3.3). NB/TSL, which does not change

with age or body size (Findley 1961), did not change with season. For those skull size variables

that do change with season, if Dehnel's phenomenon was true we would expect to see size

reduction in fall with smallest skulls in winter and largest in the summer.

Table 3.3 – Means and standard deviations for each skull variable for each season

	Spring	Summer	Fall	Winter
CBL (mm)	43.38 ± 1.75	43.09 ± 1.78	42.97 ± 1.93	42.69 ± 1.84
NB (mm)	14.21 ± 1.14	13.96 ± 1.11	14.07 ± 1.17	13.93 ± 1.10
MZW (mm)	26.96 ± 1.20	26.76 ± 1.21	26.69 ± 1.21	26.45 ± 1.24
Depth (mm)	16.61 ± 0.53	16.37 ± 0.53	16.33 ± 0.55	16.34 ± 0.50
Volume (ml)	4.32 ± 0.39	4.28 ± 0.33	4.27 ± 0.32	4.18 ± 0.33
NB/TSL	0.32 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	0.32 ± 0.02
MZW/CBL	0.62 ± 0.02	0.62 ± 0.02	0.62 ± 0.02	0.62 ± 0.02
Cranial box (mm^3)	19493 ± 1912.27	18946.17 ± 1965.31	18818.56 ± 1976.40	18538 ± 1957.34

DISCUSSION

Considering the multimodel inference on skull variables of *T.hudsonicus*, the most common

models describing all variables other than Depth and MZW/CBL included all three geographical

interactions (elevation:longitude, elevation:latitude and longitude:latitude)(table 3.1). All

variables other than Depth and MZW/CBL increased with elevation (figure 3.3). Latitude did not

show a strong relationship with most variables apart from NB and NB/TSL (figure 3.4). The

expectations based on Bergmann's rule would be that size would increase with elevation and

latitude, because these would be indicators of colder conditions. While we see the expected

relationship for elevation we do not for latitude. This could be predicted due to the nature of the

species distribution i.e. the species is found at low elevation at high latitudes and high elevation

at low latitudes. This can be seen when examining the distribution of TSL categories (figure 3.1).

Among the lower elevation populations, those at higher latitude in the north-west (Alaska and

nearby areas of Canada) tend to be larger than the eastern populations of Canada and the US. The

largest TSL values are found in very high elevation but low latitude areas in the Rocky

Mountains. Also in the east, the largest skulls are associated with the high elevation populations

of the Appalachians.

When considering longitude all variables other than MZW/CBL show a reduction to lower longitudes with smallest skulls being found in eastern North America (figure 3.5). This again can be seen as a support for Bergmann's rule given that higher elevation can be found in the western part of the distribution along the Rocky Mountains.

Although the skull data generally support a Bergmann trend, there is need for caution. It is evident from the distribution of the TSL categories in figure 3.1 that large geographic regions have similar sized skulls and such a pattern need not necessarily come about through adaptive processes - it could also come about through historical processes. Squirrels from large coherent geographical regions could derive from single refugia at the Last Glacial Maximum (LGM; Hewitt 2000) and their skull characteristics could, at least in part, reflect that common history. The locations of the LGM refugia are not precisely clear from phylogeographic studies that have been carried out to date on *T.hudsonicus*. However, according to Hope et al (2016), the eastern North American populations derive from a different refugium from the Alaskan populations, and populations in the southern Rocky Mountains and along the north Pacific coast also have a different history.

As well as examining Bergmann's rule with the skull data, it is possible to test for Allen's rule in *T.hudsonicus*. When looking at the results for nasal bones simply for latitude they might suggest support for Allen's rule where you would expect smaller extremities at higher latitudes. Both NB and NB/TSL decrease with latitude (figure 3.4). However, the response of nasal bones to elevation does not seem to support this, with an increase in both NB and NB/TSL up an elevational gradient (figure 3.3). It is possible that there are two factors at work here. Temperature might be an important factor in the reduction of the nose as the species goes further north but not as it goes up an elevational gradient. It might also be possible that the latitudinal

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trend seen is mainly being driven by elevation in the southern part of the distribution. Another possibility is that the length of nasal bones may be an adaptive response to humidity. In North America the driest areas can be found in the south-western part of the *T.hudsonicus* distribution, where the species is found at high elevation, with greater humidity in the north and east (https://nelson.wisc.edu/sage/data-and-models/atlas/maps/avgannrh/atl_avgannrh_nam.jpg). This fits with both the lengthening of the nasal bones up an elevation gradient and further south. The importance of humidity might be tested by detailed studies of the turbinate bones in the nose. One of their main function is the humidification of inhaled air and water retention of exhaled air (Jackson & Schmidt-Nielsen 1964). By increasing the length of the nasal bones, and therefore the size of the nose, the surface area of the turbinate bones is expected to be greater, potentially reducing water loss under the drier conditions. This needs to be further tested by including data on humidity in the models.

The only previous test of Bergmann's and Allen's rules in *T.hudsonicus* using skulls was limited to the Rocky Mountains and used a multivariate approach (Lindsay 1987). The results are not easily related to ours because elevation was not considered as one of the factors, and the geographical scale of the study was much more limited than ours. In our companion paper (chapter 2), we found Bergmann trends for external measurements such as total body length, consistent with what we find here with skulls. Tail length, hind foot length and ear length did not show an Allen trend, and although nose length may show an Allen trend with respect to latitude in our study here, there may be confounding issues with possible counteracting selective pressures, and caution is needed with regards demonstration of Allen's rule in *T.hudsonicus.*

At first sight our data provides indications of Dehnel's phenomenon with smaller values of most skull variables in the winter compared with other seasons (table 3.3). This includes

Volume and Cranial box which relate to brain size. However, skull depth, which provides one of the most striking decreases in skull dimensions recorded in winter in *Sorex* shrews (Pucek 1970) does not vary between summer, fall and winter. Also, spring values are systematically larger than other values at other seasons. Therefore, it is not that winter is exceptional as a season of decreased size because of response to temperature. There is also a decline from spring to summer, when there is an increase in temperature. Dehnel's phenomenon in shrews derives from changes in size of individuals; individuals get smaller in various ways in the winter, and this is believed to be an adaptive response of individuals to a decline in food supply (Churchfield et al 2012). It is possible that the decline in size in winter in *T.hudsonicus* reflects a loss of relatively large individuals from the population, leading to a reduction in average size among those individuals collected. If so, this would be interesting as a selective response to temperature change, but it would not be Dehnel's phenomenon, which is a developmental process.

The inclusion of sex as a main factor in all of the most common models and for all variables apart from MZW/CBL, with males always being bigger than females is somewhat unexpected. The species is not considered to be strongly sexually dimorphic and previously described sexual dimorphism has only been for some measurements and while males have generally been larger where a difference has been found that has not always been the case (Durrant & Hansen 1954; Layne 1954; Findley 1961; Nellis 1969; Kramm, Maki & Glime 1975). This is however strongly supported by our data and does not seem to be driven by isolated populations.

The two ratios used (NB/TSL and MZW/CBL) have previously been shown not to be impacted by the age of the specimen (Findley 1961) and were included, in part, as definitive bias-free variables. Moreover since neither are dependent on the size of the skull they should be

serve as independently variable characters. The difference between the two is striking. Only one model is well supported when it comes to explaining the variation in MZW/CBL, a model not shared with another variable. Sexual dimorphism does not appear to be important and while the interaction between season and longitude is found in the best model this is most likely to reflect the small number of samples west of the Rocky Mountains in fall and spring and lack of more suitable models, allowing the sampling bias to increase in apparent importance. NB/TSL however is a very different story. In all cases the best models are the same as those found with other variables with males showing relatively longer noses than females and the size of the nose relative to the skull decreasing slightly northwards and increasing up an elevation gradient.

The most interesting results to this study relate to elevation. From a range of skull dimensions, *T.hudsonicus* get larger with elevation and, as a further characteristic, their noses get proportionally longer with elevation. There is a likelihood that this reflects adaptive responses to temperature and humidity (with elongation of the nose being a response to humidity but not elevation in itself), but further studies would be desirable to confirm this. Microgeographical studies of a very large number of skulls such as Lindsay (1987) carried out in the Rocky Mountains would be worthwhile, but with the incorporation of elevation as a factor. A thorough phylogeographic analysis is needed to provide a historical perspective, and an integration of studies on adaptation and studies on postglacial colonization (e.g. Zamudio, Bell & Mason 2016).

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SUPPLEMENTARY MATERIAL 3

Table S3.1 – Models evaluated for the study

Model Number	Model factors
Model 1	Elevation + Latitude + Season + Sex + Longitude
Model 2	Elevation + Season + Sex + Longitude
Model 3	Elevation + Season + Longitude + Latitude
Model 4	Elevation + Latitude + Sex + Longitude
Model 5	Elevation + Latitude + Longitude
Model 6	Elevation + Season + Latitude
Model 7	Elevation + Season + Longitude
Model 8	Elevation + Longitude
Model 9	Latitude + Longitude
Model 10	Latitude + Elevation
Model 11	Elevation + Season + Latitude + Longitude + Season:Elevation
Model 12	Elevation + Season + Latitude + Longitude + Season:Longitude
Model 13	Elevation + Season + Latitude + Longitude + Season:Latitude
Model 14	Elevation + Season + Latitude + Longitude + Elevation:Longitude
Model 15	Elevation + Season + Longitude + Latitude + Latitude:Longitude
Model 16	Elevation + Season + Longitude + Latitude + Elevation:Latitude
Model 17	Elevation + Latitude + Longitude + Elevation:Longitude
Model 18	Elevation + Latitude + Longitude + Elevation:Latitude
Model 19	Elevation + Latitude + Longitude + Longitude:Latitude
Model 20	Elevation + Season + Longitude + Elevation:Longitude
Model 21	Latitude + Season + Longitude + Latitude:Longitude
Model 22	Latitude + Season + Elevation + Latitude:Elevation
Model 23	Elevation + Longitude + Elevation:Longitude
Model 24	Latitude + Longitude + Latitude:Longitude
Model 25	Latitude + Elevation + Latitude:Elevation
Model 26	Elevation + Latitude + Season + Sex + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 27	Elevation + Latitude + Season + Sex + Longitude + Elevation:Season + Season:Latitude
Model 28	Elevation + Latitude + Season + Sex + Longitude + Elevation:Latitude + Season:Latitude + Longitude:Latitude
Model 29	Elevation + Latitude + Sex + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 30	Elevation + Latitude + Season + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 31	Elevation + Latitude + Longitude + Elevation:Longitude + Elevation:Latitude + Longitude:Latitude
Model 32	Elevation + Season + Sex + Latitude + Longitude + Sex:Elevation
Model 33	Elevation + Season + Sex + Latitude + Longitude + Sex:Latitude
Model 34	Elevation + Season + Sex + Latitude + Longitude + Sex:Longitude

Model 35	Elevation + Sex + Latitude + Longitude + Sex:Elevation
Model 36	Elevation + Sex + Latitude + Longitude + Sex:Latitude
Model 37	Elevation + Sex + Latitude + Longitude + Sex:Longitude
Model 38	Elevation + Latitude + Season + Sex + Longitude + Elevation:Longitude + Elevation:Latitude
Model 39	Elevation + Latitude + Sex + Longitude + Elevation:Longitude + Elevation:Latitude
Model 40	Elevation + Latitude + Season + Longitude + Elevation:Longitude + Elevation:Latitude
Model 41	Elevation + Latitude + Longitude + Elevation:Longitude + Elevation:Latitude
Model 42	Elevation + Latitude + Season + Sex + Longitude + Latitude:Longitude + Latitude:Elevation
Model 43	Elevation + Latitude + Sex + Longitude + Latitude:Longitude + Latitude:Elevation
Model 44	Elevation + Latitude + Season + Longitude + Latitude:Longitude + Latitude:Elevation
Model 45	Elevation + Latitude + Longitude + Latitude:Longitude + Latitude:Elevation
Model 46	Elevation + Latitude + Season + Sex + Longitude + Longitude:Elevation + Longitude:Latitude
Model 47	Elevation + Latitude + Sex + Longitude + Longitude:Elevation + Longitude:Latitude
Model 48	Elevation + Latitude + Season + Longitude + Longitude:Elevation + Longitude:Latitude
Model 49	Elevation + Latitude + Longitude + Longitude:Elevation + Longitude:Latitude

Table S3.2 – Results from model selection for CBL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
29	19.72	0.00047	0.33530	-0.25590		+				0.00001	0.00391	0.00002				9	-1110.31	2238.90	0.00	0.63
43	22.39	-0.00078	0.29000	-0.23170		+					0.00351	0.00002				8	-1112.33	2240.90	1.98	0.23
47	19.20	0.00103	0.35480	-0.24990		+				0.00001	0.00386					8	-1113.68	2243.60	4.69	0.06
26	20.06	0.00044	0.32980	-0.25000	+	+				0.00001	0.00382	0.00002				12	-1109.70	2243.90	4.99	0.05
42	22.68	-0.00073	0.28540	-0.22600	+	+					0.00343	0.00002				11	-1111.48	2245.40	6.47	0.03
46	19.56	0.00099	0.34870	-0.24370	+	+				0.00001	0.00377					11	-1112.95	2248.30	9.41	0.01
28	22.70	-0.00076	0.28570	-0.22590	+	+			+		0.00343	0.00002				14	-1110.95	2250.60	11.67	0.00
31	19.18	0.00068	0.35090	-0.26110						0.00001	0.00401	0.00002				8	-1119.94	2256.10	17.20	0.00
24	20.88		0.32920	-0.23980							0.00369					5	-1124.22	2258.50	19.63	0.00
45	22.12	-0.00069	0.30140	-0.23450							0.00358	0.00002				7	-1122.31	2258.80	19.90	0.00
49	18.71	0.00119	0.36880	-0.25530						0.00001	0.00397					7	-1122.80	2259.80	20.87	0.00
19	21.06	0.00004	0.32740	-0.23580							0.00364					6	-1124.11	2260.30	21.45	0.00
30	19.17	0.00069	0.35270	-0.25790	+					0.00001	0.00398	0.00002				11	-1119.24	2260.90	22.00	0.00
44	22.11	-0.00064	0.30300	-0.23090	+						0.00354	0.00002				10	-1121.45	2263.20	24.35	0.00
21	20.73		0.33150	-0.24050	+						0.00371					8	-1123.64	2263.50	24.61	0.00
48	18.71	0.00120	0.36990	-0.25180	+					0.00001	0.00393					10	-1122.00	2264.30	25.45	0.00
15	21.05	0.00009	0.32880	-0.23210	+						0.00360					9	-1123.21	2264.70	25.80	0.00
38	41.50	-0.00221	-0.08143	-0.04476	+	+				-0.00002		0.00002				11	-1146.08	2314.60	75.69	0.00
39	41.75	-0.00227	-0.08811	-0.04633		+				-0.00002		0.00002				8	-1150.86	2317.90	79.04	0.00
33	39.10	0.00036	-0.03501	-0.04642	+	+								+		10	-1151.37	2323.10	84.19	0.00
34	39.24	0.00035	-0.05121	-0.05229	+	+									+	10	-1151.54	2323.40	84.53	0.00
1	39.72	0.00037	-0.04858	-0.04631	+	+										9	-1153.05	2324.40	85.48	0.00
36	39.27	0.00033	-0.03934	-0.04801		+								+		7	-1155.85	2325.90	86.97	0.00
32	39.69	0.00040	-0.04882	-0.04631	+	+							+			10	-1152.88	2326.10	87.20	0.00
37	39.42	0.00031	-0.05751	-0.05473		+									+	7	-1155.97	2326.10	87.22	0.00
27	37.94	0.00054	-0.01727	-0.04791	+	+	+		+							15	-1148.09	2326.90	88.03	0.00
4	39.96	0.00033	-0.05444	-0.04786		+										6	-1157.92	2328.00	89.07	0.00
35	39.93	0.00036	-0.05462	-0.04785		+								+		7	-1157.79	2329.80	90.86	0.00
2	38.28	0.00064		-0.03545	+	+										8	-1159.33	2334.90	95.99	0.00
40	41.49	-0.00205	-0.07454	-0.04404	+					-0.00002		0.00002				10	-1157.70	2335.70	96.84	0.00
14	40.88	-0.00161	-0.05612	-0.04140	+					-0.00002						9	-1159.29	2336.90	97.95	0.00
41	41.78	-0.00212	-0.08276	-0.04609						-0.00001		0.00002				7	-1161.41	2337.00	98.09	0.00
17	41.17	-0.00167	-0.06389	-0.04344						-0.00002						6	-1163.09	2338.30	99.41	0.00
16	40.87	-0.00065	-0.07189	-0.04833	+							0.00002				9	-1160.56	2339.40	100.51	0.00
18	41.16	-0.00072	-0.07981	-0.05028								0.00002				6	-1164.22	2340.60	101.67	0.00
13	39.09	0.00038	-0.03136	-0.04729	+				+							11	-1160.18	2342.80	103.88	0.00
3	39.83	0.00038	-0.04449	-0.04570	+											8	-1163.63	2343.50	104.59	0.00
5	40.10	0.00033	-0.05183	-0.04768												5	-1167.42	2344.90	106.04	0.00
12	38.67	0.00038	-0.04068	-0.05568	+			+								11	-1161.59	2345.60	106.69	0.00

11	39.75	0.00045	-0.04436	-0.04537	+		+								11	-1162.56	2347.50	108.64	0.00
20	38.95	-0.00059		-0.03135	+					-0.00001					8	-1166.90	2350.00	111.13	0.00
7	38.50	0.00063		-0.03574	+										7	-1168.76	2351.70	112.80	0.00
9	41.13		-0.08771	-0.05725											4	-1173.69	2355.40	116.54	0.00
23	38.96	-0.00042		-0.03279						-0.00001					5	-1173.79	2357.70	118.78	0.00
8	38.58	0.00061		-0.03642											4	-1175.09	2358.20	119.34	0.00
6	38.72	0.00110	0.06014		+										7	-1215.11	2444.40	205.50	0.00
22	38.67	0.00115	0.06113		+					0.00000					8	-1215.10	2446.40	207.53	0.00
10	39.46	0.00102	0.05255												4	-1227.59	2463.30	224.35	0.00
25	39.34	0.00114	0.05525							0.00000					5	-1227.55	2465.20	226.30	0.00

Table S3.3 – Results from model selection for MZW; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
29	11.86	0.00079	0.22210	-0.15270		+				0.00001	0.00237	0.00001				9	-892.13	1802.50	0.00	0.28
47	11.67	0.00101	0.22940	-0.15020		+				0.00001	0.00235					8	-893.20	1802.60	0.09	0.26
46	12.31	0.00094	0.21810	-0.14120	+	+				0.00001	0.00220					11	-890.65	1803.70	1.18	0.15
26	12.49	0.00073	0.21120	-0.14380	+	+				0.00001	0.00223	0.00001				12	-889.68	1803.90	1.31	0.14
43	13.86	-0.00014	0.18800	-0.13470		+					0.00207	0.00001				8	-894.35	1804.90	2.37	0.08
42	14.33	-0.00009	0.17990	-0.12700	+	+					0.00195	0.00001				11	-891.44	1805.30	2.74	0.07
28	14.61	-0.00012	0.17430	-0.12750	+	+			+		0.00197	0.00001				14	-890.49	1809.60	7.09	0.01
49	11.41	0.00110	0.23710	-0.15330						0.00001	0.00241					7	-899.73	1813.60	11.09	0.00
31	11.58	0.00090	0.23060	-0.15570						0.00001	0.00243	0.00001				8	-898.87	1814.00	11.41	0.00
19	13.43	0.00011	0.20140	-0.13660							0.00212					6	-901.67	1815.50	12.93	0.00
48	11.83	0.00106	0.23040	-0.14610	+					0.00001	0.00230					10	-897.57	1815.50	12.93	0.00
30	11.98	0.00087	0.22430	-0.14850	+					0.00001	0.00232	0.00001				11	-896.79	1816.00	13.45	0.00
24	12.95		0.20610	-0.14740							0.00227					5	-903.21	1816.50	13.97	0.00
15	13.72	0.00016	0.19690	-0.13020	+						0.00203					9	-899.18	1816.60	14.09	0.00
45	13.73	-0.00010	0.19410	-0.13630							0.00211	0.00000				7	-901.37	1816.90	14.38	0.00
44	14.01	-0.00004	0.18990	-0.13000	+						0.00201	0.00000				10	-898.90	1818.10	15.59	0.00
21	13.16		0.20160	-0.14490	+						0.00223					8	-901.92	1820.10	17.52	0.00
34	23.80	0.00030	-0.01688	-0.03034	+	+									+	10	-915.63	1851.60	49.06	0.00
38	24.91	-0.00081	-0.02756	-0.02463	+	+				-0.00001		0.00001				11	-914.84	1852.10	49.54	0.00
33	23.82	0.00032	-0.00715	-0.02576	+	+								+		10	-916.41	1853.20	50.61	0.00
1	24.17	0.00032	-0.01494	-0.02572	+	+										9	-917.50	1853.30	50.73	0.00
2	23.73	0.00040		-0.02237	+	+										8	-918.77	1853.80	51.21	0.00
32	24.16	0.00033	-0.01506	-0.02572	+	+							+			10	-917.41	1855.20	52.62	0.00
27	24.09	0.00033	-0.01472	-0.02620	+	+	+		+							15	-912.22	1855.20	52.66	0.00
37	23.96	0.00027	-0.02264	-0.03249		+									+	7	-922.43	1859.00	56.49	0.00
39	25.15	-0.00088	-0.03369	-0.02609		+				-0.00001		0.00001				8	-922.11	1860.40	57.91	0.00
36	23.99	0.00028	-0.01160	-0.02722		+								+		7	-923.48	1861.10	58.60	0.00
4	24.39	0.00028	-0.02047	-0.02717		+										6	-924.87	1861.90	59.33	0.00
35	24.38	0.00030	-0.02056	-0.02717		+							+			7	-924.81	1863.80	61.25	0.00
14	24.74	-0.00059	-0.01847	-0.02352	+					-0.00001						9	-923.91	1866.10	63.56	0.00
20	24.10	-0.00024		-0.02025	+					-0.00001						8	-925.66	1867.50	65.00	0.00
40	24.93	-0.00072	-0.02400	-0.02430	+					-0.00001		0.00001				10	-923.61	1867.60	65.01	0.00
7	23.87	0.00040		-0.02255	+											7	-926.76	1867.70	65.14	0.00
12	24.57	0.00036	-0.00945	-0.02021	+			+								11	-922.71	1867.80	65.30	0.00
3	24.25	0.00032	-0.01293	-0.02545	+											8	-925.83	1867.90	65.33	0.00
16	24.62	-0.00004	-0.02246	-0.02634	+							0.00001				9	-925.03	1868.30	65.80	0.00
13	24.23	0.00033	-0.01332	-0.02581	+				+							11	-923.67	1869.70	67.20	0.00
17	24.99	-0.00065	-0.02508	-0.02520						-0.00001						6	-930.04	1872.20	69.68	0.00
11	24.21	0.00034	-0.01261	-0.02544	+		+									11	-925.43	1873.30	70.73	0.00

41	25.18	-0.00079	-0.03103	-0.02603						-0.00001		0.00001				7	-929.69	1873.50	71.01	0.00
5	24.49	0.00028	-0.01930	-0.02714												5	-932.00	1874.10	71.54	0.00
18	24.87	-0.00010	-0.02935	-0.02805								0.00001				6	-931.12	1874.40	71.83	0.00
8	23.93	0.00038		-0.02292												4	-934.25	1876.60	74.02	0.00
23	24.12	-0.00015		-0.02103						0.00000						5	-933.50	1877.10	74.56	0.00
9	25.35		-0.04976	-0.03535												4	-941.45	1891.00	88.43	0.00
6	23.66	0.00072	0.04492		+											7	-960.46	1935.10	132.54	0.00
22	23.46	0.00093	0.04930		+							0.00000				8	-960.20	1936.60	134.08	0.00
10	24.14	0.00067	0.03974													4	-973.70	1955.50	152.92	0.00
25	23.90	0.00092	0.04516									-0.00001				5	-973.33	1956.80	154.22	0.00

Table S3.4 – Results from model selection for NB; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
43	4.57	0.00082	0.12910	-0.10960		+					0.00168	-0.00002				8	-802.88	1622.00	0.00	0.33
29	5.71	0.00029	0.10980	-0.09933		+				-0.00001	0.00151	-0.00002				9	-801.93	1622.10	0.16	0.30
42	4.17	0.00087	0.13890	-0.11030	+	+					0.00172	-0.00002				11	-800.36	1623.10	1.14	0.18
26	5.32	0.00036	0.11920	-0.09976	+	+				-0.00001	0.00155	-0.00002				12	-799.45	1623.40	1.39	0.16
28	4.45	0.00088	0.13330	-0.10830	+	+			+		0.00168	-0.00002				14	-799.48	1627.60	5.63	0.02
47	6.14	-0.00016	0.09386	-0.10400		+				0.00000	0.00154					8	-807.60	1631.40	9.44	0.00
44	3.95	0.00091	0.14630	-0.11220	+						0.00176	-0.00002				10	-805.95	1632.20	10.26	0.00
46	5.76	-0.00010	0.10330	-0.10480	+	+				0.00000	0.00158					11	-805.24	1632.90	10.91	0.00
30	4.93	0.00048	0.12960	-0.10320	+					0.00000	0.00162	-0.00002				11	-805.30	1633.00	11.02	0.00
45	4.49	0.00086	0.13380	-0.11050							0.00170	-0.00002				7	-809.62	1633.40	11.42	0.00
31	5.48	0.00040	0.11700	-0.10150						0.00000	0.00155	-0.00002				8	-808.91	1634.00	12.05	0.00
15	5.11	0.00012	0.11800	-0.11070	+						0.00170					9	-811.46	1641.20	19.21	0.00
24	5.30		0.10910	-0.11630							0.00174					5	-815.95	1642.00	20.00	0.00
19	5.63	0.00008	0.10580	-0.10900							0.00163					6	-815.00	1642.10	20.13	0.00
48	5.37	0.00000	0.11350	-0.10860	+					0.00000	0.00166					10	-811.42	1643.20	21.19	0.00
21	4.70		0.12140	-0.12180	+						0.00185					8	-813.52	1643.30	21.27	0.00
49	5.93	-0.00007	0.10060	-0.10650						0.00000	0.00159					7	-814.94	1644.10	22.06	0.00
39	14.21	-0.00078	-0.05369	-0.01850		+				-0.00002		-0.00002				8	-818.69	1653.60	31.61	0.00
38	14.00	-0.00073	-0.04750	-0.01687	+	+				-0.00002		-0.00002				11	-815.99	1654.40	32.40	0.00
40	14.01	-0.00065	-0.04435	-0.01649	+					-0.00002		-0.00002				10	-823.16	1666.70	44.66	0.00
41	14.24	-0.00070	-0.05113	-0.01835						-0.00002		-0.00002				7	-826.29	1666.80	44.77	0.00
37	13.73	0.00020	-0.06738	-0.02908		+									+	7	-829.71	1673.60	51.60	0.00
34	13.54	0.00024	-0.06173	-0.02751	+	+									+	10	-827.41	1675.20	53.18	0.00
4	14.09	0.00021	-0.06551	-0.02459		+										6	-832.05	1676.20	54.23	0.00
35	14.05	0.00025	-0.06585	-0.02460		+							+			7	-831.09	1676.40	54.36	0.00
1	13.90	0.00025	-0.05990	-0.02313	+	+										9	-829.66	1677.60	55.61	0.00
32	13.86	0.00030	-0.06031	-0.02316	+	+							+			10	-828.71	1677.80	55.77	0.00
17	14.95	-0.00123	-0.07313	-0.02149						-0.00001						6	-832.86	1677.90	55.87	0.00
14	14.74	-0.00120	-0.06683	-0.01977	+					-0.00001						9	-829.91	1678.10	56.11	0.00
36	14.09	0.00021	-0.06562	-0.02459		+								+		7	-832.05	1678.30	56.27	0.00
33	13.91	0.00025	-0.06017	-0.02313	+	+								+		10	-829.66	1679.70	57.67	0.00
27	13.61	0.00028	-0.05497	-0.02351	+	+	+		+							15	-825.56	1681.90	59.88	0.00
18	13.56	0.00083	-0.04768	-0.02285								-0.00001				6	-836.16	1684.40	62.46	0.00
16	13.32	0.00089	-0.04105	-0.02108	+							-0.00001				9	-833.30	1684.90	62.89	0.00
5	14.18	0.00021	-0.06425	-0.02444												5	-839.33	1688.80	66.76	0.00
3	13.96	0.00026	-0.05798	-0.02277	+											8	-836.58	1689.40	67.39	0.00
13	13.79	0.00026	-0.05503	-0.02315	+				+							11	-834.06	1690.50	68.53	0.00
12	13.58	0.00028	-0.05468	-0.02490	+			+								11	-834.84	1692.10	70.10	0.00
11	13.95	0.00027	-0.05800	-0.02279	+		+									11	-835.94	1694.30	72.30	0.00

9	14.83		-0.08704	-0.03049										4	-846.41	1700.90	78.89	0.00
2	12.13	0.00060		-0.00969	+	+								8	-855.44	1727.10	105.11	0.00
20	12.43	0.00004		-0.00779	+				-0.00001					8	-859.35	1734.90	112.92	0.00
7	12.23	0.00059		-0.00976	+									7	-860.38	1734.90	112.95	0.00
22	12.32	0.00170	0.01789		+						-0.00003			8	-863.75	1743.70	121.73	0.00
8	12.30	0.00056		-0.01037										4	-871.33	1750.70	128.73	0.00
23	12.43	0.00021		-0.00915					0.00000					5	-870.93	1752.00	129.96	0.00
25	12.69	0.00170	0.01455								-0.00003			5	-874.52	1759.10	137.13	0.00
6	13.38	0.00062	-0.00523		+									7	-873.69	1761.50	139.56	0.00
10	13.82	0.00056	-0.01012											4	-885.17	1778.40	156.40	0.00

Table S3.5 – Results from model selection for Depth; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
32	16.50	-0.00001	-0.02686	-0.00962	+	+							+			10	-461.92	944.20	0.00	0.52
34	16.35	-0.00006	-0.02751	-0.01197	+	+									+	10	-462.76	945.90	1.68	0.22
1	16.54	-0.00005	-0.02646	-0.00959	+	+										9	-464.73	947.70	3.55	0.09
42	16.32	-0.00026	-0.02332	-0.01434	+	+					0.00008	0.00000				11	-463.44	949.30	5.12	0.04
38	16.70	-0.00015	-0.03180	-0.01044	+	+				0.00000		0.00001				11	-463.46	949.30	5.15	0.04
33	16.58	-0.00005	-0.02728	-0.00959	+	+								+		10	-464.68	949.70	5.52	0.03
26	15.82	-0.00004	-0.01486	-0.01889	+	+				0.00000	0.00016	0.00001				12	-462.97	950.40	6.25	0.02
46	15.71	0.00010	-0.01058	-0.01738	+	+				0.00000	0.00014					11	-464.31	951.00	6.84	0.02
35	16.67	-0.00004	-0.03103	-0.01081		+							+			7	-469.40	953.00	8.80	0.01
37	16.50	-0.00009	-0.03182	-0.01343		+									+	7	-469.77	953.70	9.53	0.00
27	16.62	-0.00006	-0.02835	-0.00981	+	+	+		+							15	-462.10	955.00	10.78	0.00
28	16.33	-0.00026	-0.02369	-0.01473	+	+			+		0.00009	0.00000				14	-463.40	955.50	11.28	0.00
4	16.70	-0.00008	-0.03066	-0.01080		+										6	-472.13	956.40	12.21	0.00
43	16.02	-0.00030	-0.01841	-0.02006		+					0.00017	0.00000				8	-470.16	956.50	12.37	0.00
29	15.37	0.00000	-0.00729	-0.02600		+				0.00000	0.00027	0.00001				9	-469.29	956.90	12.69	0.00
39	16.87	-0.00019	-0.03624	-0.01166		+				0.00000		0.00001				8	-470.76	957.70	13.57	0.00
47	15.25	0.00014	-0.00265	-0.02446		+				0.00000	0.00025					8	-470.79	957.80	13.63	0.00
36	16.72	-0.00008	-0.03104	-0.01079		+								+		7	-472.12	958.40	14.23	0.00
3	16.58	-0.00005	-0.02544	-0.00940	+											8	-471.54	959.30	15.12	0.00
16	16.76	-0.00023	-0.03028	-0.00984	+							0.00000				9	-470.71	959.70	15.52	0.00
15	15.93	-0.00006	-0.01247	-0.01587	+						0.00013					9	-471.14	960.60	16.39	0.00
12	16.60	-0.00006	-0.02715	-0.01018	+			+								11	-469.24	960.90	16.71	0.00
21	16.11		-0.01395	-0.01087	+						0.00006					8	-472.35	960.90	16.74	0.00
44	16.17	-0.00023	-0.01840	-0.01563	+						0.00011	0.00000				10	-470.40	961.10	16.95	0.00
14	16.54	0.00003	-0.02501	-0.00957	+					0.00000						9	-471.49	961.30	17.07	0.00
40	16.70	-0.00010	-0.03000	-0.01024	+					0.00000		0.00000				10	-470.50	961.30	17.16	0.00
30	15.56	0.00004	-0.00809	-0.02121	+					0.00000	0.00020	0.00001				11	-469.69	961.80	17.61	0.00
48	15.46	0.00016	-0.00429	-0.01979	+					0.00000	0.00019					10	-470.78	961.90	17.73	0.00
11	16.59	-0.00005	-0.02561	-0.00933	+		+									11	-470.28	963.00	18.80	0.00
13	16.61	-0.00005	-0.02615	-0.00943	+				+							11	-471.50	965.40	21.24	0.00
19	15.71	-0.00010	-0.00922	-0.02102							0.00020					6	-477.47	967.10	22.89	0.00
5	16.75	-0.00008	-0.03002	-0.01072												5	-478.50	967.10	22.92	0.00
18	16.95	-0.00028	-0.03514	-0.01117								0.00000				6	-477.59	967.30	23.14	0.00
31	15.21	0.00007	-0.00268	-0.02753						0.00000	0.00030	0.00001				8	-475.58	967.40	23.20	0.00
45	15.96	-0.00028	-0.01530	-0.02077							0.00019	0.00000				7	-476.69	967.60	23.38	0.00
49	15.10	0.00020	0.00150	-0.02609						0.00000	0.00029					7	-476.82	967.80	23.64	0.00
41	16.88	-0.00014	-0.03482	-0.01158						0.00000		0.00001				7	-477.37	968.90	24.72	0.00
17	16.71	-0.00001	-0.02957	-0.01089						0.00000						6	-478.45	969.00	24.85	0.00
9	16.50		-0.02112	-0.00830												4	-481.79	971.60	27.45	0.00

24	16.11		-0.01309	-0.01182						0.00007					5	-481.64	973.40	29.19	0.00
2	15.76	0.00010		-0.00364	+	+									8	-480.30	976.80	32.64	0.00
20	15.67	0.00048		-0.00511	+				0.00000						8	-484.17	984.60	40.38	0.00
7	15.82	0.00010		-0.00368	+										7	-485.71	985.60	41.41	0.00
6	16.36	0.00010	-0.00426		+										7	-490.78	995.70	51.56	0.00
22	16.35	0.00012	-0.00386		+						0.00000				8	-490.78	997.80	53.60	0.00
23	15.69	0.00057		-0.00596					0.00000						5	-497.34	1004.80	60.59	0.00
8	15.88	0.00008		-0.00416											4	-499.78	1007.60	63.44	0.00
10	16.62	0.00007	-0.00692												4	-505.03	1018.10	73.95	0.00
25	16.58	0.00012	-0.00588								0.00000				5	-504.98	1020.10	75.87	0.00

Table S3.6 – Results from model selection for Volume; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
47	0.72	0.00045	0.05099	-0.03475		+				0.00000	0.00051					8	-98.72	213.70	0.00	0.38
29	0.78	0.00038	0.04902	-0.03552		+				0.00000	0.00052	0.00000				9	-97.85	214.00	0.32	0.33
46	1.02	0.00042	0.04550	-0.03190	+	+				0.00000	0.00046					11	-96.54	215.50	1.84	0.15
26	1.06	0.00035	0.04364	-0.03270	+	+				0.00000	0.00047	0.00000				12	-95.71	215.90	2.26	0.12
43	1.63	-0.00003	0.03471	-0.02779		+					0.00040	0.00000				8	-102.31	220.90	7.18	0.01
42	1.88	-0.00003	0.02992	-0.02519	+	+					0.00035	0.00000				11	-99.50	221.40	7.77	0.01
28	2.00	-0.00003	0.02740	-0.02531	+	+			+		0.00035	0.00000				14	-99.09	226.90	13.22	0.00
34	3.47	0.00005	-0.00554	-0.00908	+	+									+	10	-104.30	229.00	15.29	0.00
32	3.60	0.00008	-0.00457	-0.00712	+	+								+		10	-105.90	232.20	18.49	0.00
37	3.47	0.00005	-0.00620	-0.00941		+									+	7	-109.21	232.60	18.92	0.00
49	0.59	0.00048	0.05471	-0.03626						0.00000	0.00054					7	-109.25	232.70	19.01	0.00
31	0.64	0.00042	0.05298	-0.03696						0.00000	0.00055	0.00000				8	-108.58	233.40	19.72	0.00
1	3.62	0.00006	-0.00441	-0.00709	+	+										9	-108.03	234.40	20.68	0.00
2	3.49	0.00008		-0.00611	+	+										8	-109.22	234.70	21.01	0.00
33	3.57	0.00006	-0.00321	-0.00708	+	+							+			10	-107.71	235.80	22.12	0.00
48	0.79	0.00045	0.05121	-0.03420	+					0.00000	0.00051					10	-108.16	236.70	23.01	0.00
30	0.83	0.00040	0.04960	-0.03493	+					0.00000	0.00051	0.00000				11	-107.52	237.50	23.82	0.00
38	3.66	0.00005	-0.00620	-0.00745	+	+				0.00000		0.00000				11	-107.53	237.50	23.84	0.00
24	1.39		0.04010	-0.03085							0.00045					5	-113.91	237.90	24.23	0.00
35	3.61	0.00008	-0.00511	-0.00719		+							+			7	-112.06	238.30	24.63	0.00
19	1.48	0.00002	0.03917	-0.02888							0.00042					6	-113.43	239.00	25.32	0.00
4	3.64	0.00006	-0.00495	-0.00716		+										6	-113.89	239.90	26.24	0.00
36	3.56	0.00006	-0.00330	-0.00715		+								+		7	-113.31	240.80	27.14	0.00
45	1.53	-0.00002	0.03801	-0.02887							0.00042	0.00000				7	-113.34	240.90	27.19	0.00
21	1.55		0.03714	-0.02937	+						0.00042					8	-112.53	241.30	27.61	0.00
15	1.65	0.00003	0.03619	-0.02692	+						0.00039					9	-111.82	241.90	28.27	0.00
39	3.68	0.00004	-0.00677	-0.00750		+				0.00000		0.00000				8	-113.42	243.10	29.39	0.00
27	3.72	0.00005	-0.00698	-0.00731	+	+	+		+							15	-106.26	243.30	29.66	0.00
44	1.70	-0.00001	0.03500	-0.02691	+						0.00038	0.00000				10	-111.73	243.80	30.15	0.00
7	3.55	0.00008		-0.00612	+											7	-122.34	258.90	45.20	0.00
3	3.65	0.00006	-0.00357	-0.00692	+											8	-121.59	259.40	45.75	0.00
20	3.51	0.00017		-0.00645	+					0.00000						8	-122.13	260.50	46.81	0.00
16	3.71	0.00000	-0.00506	-0.00704	+							0.00000				9	-121.39	261.10	47.41	0.00
14	3.63	0.00011	-0.00328	-0.00703	+					0.00000						9	-121.54	261.40	47.70	0.00
5	3.68	0.00006	-0.00441	-0.00708												5	-125.67	261.40	47.76	0.00
12	3.79	0.00006	-0.00308	-0.00527	+			+								11	-119.54	261.50	47.85	0.00
8	3.55	0.00008		-0.00612												4	-126.91	261.90	48.21	0.00
40	3.68	0.00007	-0.00491	-0.00724	+					0.00000		0.00000				10	-121.27	262.90	49.23	0.00
18	3.74	0.00000	-0.00597	-0.00721								0.00000				6	-125.46	263.10	49.37	0.00

23	3.51	0.00018		-0.00649						0.00000						5	-126.62	263.30	49.66	0.00
17	3.65	0.00010	-0.00415	-0.00718						0.00000						6	-125.63	263.40	49.71	0.00
13	3.71	0.00006	-0.00509	-0.00699	+				+							11	-120.90	264.20	50.57	0.00
41	3.71	0.00006	-0.00581	-0.00739						0.00000		0.00000				7	-125.34	264.90	51.20	0.00
11	3.65	0.00006	-0.00359	-0.00688	+		+									11	-121.39	265.20	51.54	0.00
9	3.84		-0.01040	-0.00872												4	-129.50	267.10	53.39	0.00
6	3.50	0.00017	0.01197		+											7	-148.86	311.90	98.22	0.00
22	3.42	0.00025	0.01382		+							0.00000				8	-148.41	313.10	99.39	0.00
10	3.58	0.00016	0.01091													4	-155.89	319.80	106.16	0.00
25	3.49	0.00025	0.01293									0.00000				5	-155.38	320.90	107.18	0.00

Table S3.7 – Results from model selection for MZW/CBL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
12	0.63	0.00000	0.00037	0.00027	+			+								11	1744.93	-3467.40	0.00	0.32
10	0.61	0.00000	0.00019													4	1736.60	-3465.10	2.30	0.10
25	0.61	0.00001	0.00030									0.00000				5	1737.35	-3464.60	2.84	0.08
18	0.60	0.00001	0.00047	0.00006								0.00000				6	1738.23	-3464.30	3.10	0.07
5	0.61	0.00000	0.00029	0.00005												5	1737.11	-3464.10	3.31	0.06
9	0.62		0.00012	0.00000												4	1736.02	-3464.00	3.47	0.06
45	0.61	0.00001	0.00041	0.00009							0.00000	0.00000				7	1738.24	-3462.30	5.13	0.02
41	0.60	0.00001	0.00047	0.00006						0.00000		0.00000				7	1738.24	-3462.30	5.15	0.02
17	0.61	0.00000	0.00031	0.00004						0.00000						6	1737.20	-3462.30	5.17	0.02
8	0.62	0.00000		-0.00002												4	1735.13	-3462.20	5.25	0.02
19	0.62	0.00000	0.00017	0.00011							0.00000					6	1737.14	-3462.20	5.29	0.02
4	0.61	0.00000	0.00029	0.00005		+										6	1737.11	-3462.10	5.35	0.02
24	0.61		0.00024	-0.00005							0.00000					5	1736.05	-3462.00	5.45	0.02
6	0.61	0.00000	0.00020		+											7	1737.64	-3461.10	6.34	0.01
16	0.60	0.00001	0.00052	0.00008	+							0.00000				9	1739.57	-3460.90	6.58	0.01
3	0.61	0.00000	0.00033	0.00006	+											8	1738.38	-3460.50	6.90	0.01
22	0.61	0.00001	0.00032		+							0.00000				8	1738.38	-3460.50	6.91	0.01
43	0.61	0.00001	0.00041	0.00009		+					0.00000	0.00000				8	1738.25	-3460.30	7.16	0.01
37	0.61	0.00000	0.00028	0.00003		+									+	7	1737.23	-3460.30	7.16	0.01
39	0.60	0.00001	0.00047	0.00006		+				0.00000		0.00000				8	1738.25	-3460.30	7.18	0.01
31	0.61	0.00001	0.00040	0.00010						0.00000	0.00000	0.00000				8	1738.24	-3460.30	7.18	0.01
49	0.61	0.00000	0.00028	0.00006						0.00000	0.00000					7	1737.21	-3460.20	7.21	0.01
23	0.62	0.00000		-0.00001						0.00000						5	1735.15	-3460.20	7.25	0.01
35	0.61	0.00000	0.00029	0.00005		+							+			7	1737.13	-3460.10	7.35	0.01
36	0.61	0.00000	0.00029	0.00005		+								+		7	1737.12	-3460.10	7.39	0.01
44	0.61	0.00001	0.00029	0.00019	+						0.00000	0.00000				10	1739.69	-3459.00	8.41	0.01
15	0.62	0.00000	0.00005	0.00020	+						0.00000					9	1738.57	-3458.80	8.59	0.00
40	0.60	0.00001	0.00052	0.00008	+					0.00000		0.00000				10	1739.58	-3458.80	8.64	0.00
11	0.61	0.00000	0.00034	0.00005	+	+										11	1740.58	-3458.70	8.70	0.00
14	0.61	0.00001	0.00035	0.00005	+					0.00000						9	1738.48	-3458.70	8.77	0.00
1	0.61	0.00000	0.00033	0.00006	+	+										9	1738.38	-3458.50	8.96	0.00
29	0.61	0.00001	0.00041	0.00010		+				0.00000	0.00000	0.00000				9	1738.25	-3458.20	9.22	0.00
47	0.61	0.00000	0.00028	0.00005		+				0.00000	0.00000					8	1737.21	-3458.20	9.25	0.00
21	0.62		0.00011	0.00002	+						0.00000					8	1737.05	-3457.90	9.56	0.00
7	0.62	0.00000		-0.00002	+											7	1736.00	-3457.80	9.62	0.00
30	0.62	0.00001	0.00023	0.00022	+					0.00000	0.00000	0.00000				11	1739.72	-3457.00	10.43	0.00
42	0.61	0.00001	0.00029	0.00019	+	+					0.00000	0.00000				11	1739.69	-3457.00	10.48	0.00
48	0.62	0.00000	0.00010	0.00018	+					0.00000	0.00000					10	1738.58	-3456.80	10.63	0.00
38	0.60	0.00001	0.00052	0.00008	+	+				0.00000		0.00000				11	1739.58	-3456.70	10.71	0.00

34	0.61	0.00000	0.00033	0.00004	+	+									+	10	1738.45	-3456.60	10.89	0.00
32	0.61	0.00000	0.00034	0.00006	+	+							+			10	1738.40	-3456.40	11.00	0.00
33	0.61	0.00000	0.00032	0.00006	+	+								+		10	1738.39	-3456.40	11.01	0.00
20	0.62	0.00000		-0.00001	+					0.00000						8	1736.02	-3455.80	11.64	0.00
2	0.62	0.00000		-0.00002	+	+										8	1736.01	-3455.80	11.65	0.00
27	0.63	0.00000	0.00001	0.00006	+	+	+		+							15	1743.15	-3455.50	11.91	0.00
13	0.62	0.00000	0.00020	0.00007	+				+							11	1738.69	-3455.00	12.49	0.00
26	0.62	0.00001	0.00023	0.00022	+	+				0.00000	0.00000	0.00000				12	1739.72	-3454.90	12.51	0.00
46	0.62	0.00000	0.00010	0.00018	+	+				0.00000	0.00000					11	1738.58	-3454.70	12.70	0.00
28	0.62	0.00001	0.00018	0.00019	+	+			+	0.00000	0.00000					14	1739.88	-3451.10	16.35	0.00

Table S3.8 – Results from model selection for NB/TSL; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
29	0.27	0.00000	0.00041	-0.00065		+				0.00000	0.00001	0.00000				9	1773.66	-3529.00	0.00	0.41
26	0.27	0.00000	0.00058	-0.00067	+	+				0.00000	0.00001	0.00000				12	1775.86	-3527.20	1.81	0.17
39	0.33	-0.00001	-0.00062	-0.00014		+				0.00000		0.00000				8	1771.72	-3527.20	1.82	0.16
31	0.27	0.00000	0.00050	-0.00068						0.00000	0.00001	0.00000				8	1770.94	-3525.70	3.38	0.08
30	0.26	0.00000	0.00070	-0.00071	+					0.00000	0.00001	0.00000				11	1773.77	-3525.10	3.91	0.06
38	0.32	0.00000	-0.00053	-0.00012	+	+				0.00000		0.00000				11	1773.73	-3525.00	3.99	0.06
41	0.33	0.00000	-0.00059	-0.00014						0.00000		0.00000				7	1768.77	-3523.40	5.68	0.02
43	0.23	0.00002	0.00116	-0.00105		+					0.00002	0.00000				8	1769.61	-3523.00	6.05	0.02
40	0.32	0.00000	-0.00050	-0.00011	+					0.00000		0.00000				10	1771.27	-3522.20	6.85	0.01
42	0.22	0.00002	0.00135	-0.00109	+	+					0.00002	0.00000				11	1771.85	-3521.30	7.75	0.01
45	0.23	0.00002	0.00121	-0.00107							0.00002	0.00000				7	1767.23	-3520.30	8.76	0.01
44	0.22	0.00002	0.00143	-0.00111	+						0.00002	0.00000				10	1770.11	-3519.90	9.17	0.00
28	0.22	0.00002	0.00126	-0.00103	+	+			+		0.00002	0.00000				14	1772.44	-3516.20	12.82	0.00
18	0.31	0.00002	-0.00053	-0.00022								0.00000				6	1759.98	-3507.80	21.20	0.00
16	0.31	0.00002	-0.00044	-0.00019	+							0.00000				9	1762.06	-3505.80	23.20	0.00
47	0.28	-0.00001	-0.00004	-0.00080		+				0.00000	0.00001					8	1759.95	-3503.70	25.37	0.00
46	0.28	-0.00001	0.00014	-0.00083	+	+				0.00000	0.00001					11	1762.17	-3501.90	27.11	0.00
24	0.25		0.00052	-0.00115							0.00002					5	1755.24	-3500.40	28.65	0.00
19	0.26	0.00000	0.00047	-0.00103							0.00001					6	1755.88	-3499.60	29.40	0.00
49	0.28	-0.00001	0.00005	-0.00083						0.00000	0.00001					7	1756.87	-3499.60	29.47	0.00
15	0.25	0.00000	0.00069	-0.00108	+						0.00002					9	1758.82	-3499.40	29.68	0.00
48	0.27	-0.00001	0.00026	-0.00088	+					0.00000	0.00001					10	1759.79	-3499.20	29.81	0.00
21	0.24		0.00074	-0.00124	+						0.00002					8	1757.54	-3498.90	30.18	0.00
17	0.35	-0.00002	-0.00119	-0.00023						0.00000						6	1754.24	-3496.30	32.69	0.00
14	0.34	-0.00002	-0.00110	-0.00020	+					0.00000						9	1756.73	-3495.20	33.86	0.00
37	0.33	0.00000	-0.00111	-0.00033		+									+	7	1754.38	-3494.60	34.46	0.00
4	0.33	0.00000	-0.00108	-0.00027		+										6	1753.26	-3494.40	34.66	0.00
35	0.33	0.00000	-0.00108	-0.00027		+							+			7	1753.87	-3493.60	35.47	0.00
36	0.34	0.00000	-0.00116	-0.00027		+								+		7	1753.72	-3493.30	35.77	0.00
34	0.33	0.00000	-0.00102	-0.00031	+	+									+	10	1756.14	-3491.90	37.11	0.00
22	0.30	0.00003	0.00009		+							0.00000				8	1754.01	-3491.80	37.24	0.00
1	0.33	0.00000	-0.00100	-0.00025	+	+										9	1754.96	-3491.60	37.40	0.00
32	0.33	0.00000	-0.00100	-0.00025	+	+							+			10	1755.56	-3490.80	38.26	0.00
33	0.33	0.00000	-0.00107	-0.00025	+	+								+		10	1755.40	-3490.40	38.60	0.00
5	0.33	0.00000	-0.00107	-0.00027												5	1750.22	-3490.30	38.70	0.00
3	0.33	0.00000	-0.00097	-0.00025	+											8	1752.39	-3488.60	40.49	0.00
25	0.31	0.00003	0.00005									0.00000				5	1748.78	-3487.50	41.57	0.00
9	0.34		-0.00133	-0.00034												4	1747.44	-3486.80	42.22	0.00
12	0.33	0.00000	-0.00093	-0.00027	+			+								11	1753.41	-3484.40	44.64	0.00

13	0.33	0.00000	-0.00093	-0.00025	+				+						11	1753.28	-3484.10	44.91	0.00
11	0.33	0.00000	-0.00098	-0.00025	+		+								11	1752.74	-3483.10	45.98	0.00
27	0.33	0.00000	-0.00095	-0.00025	+	+	+		+						15	1756.84	-3482.90	46.13	0.00
6	0.33	0.00001	-0.00041		+										7	1739.22	-3464.30	64.78	0.00
10	0.33	0.00001	-0.00048												4	1733.01	-3457.90	71.09	0.00
2	0.30	0.00001		-0.00003	+	+									8	1733.97	-3451.70	77.34	0.00
7	0.30	0.00001		-0.00003	+										7	1732.29	-3450.40	78.63	0.00
20	0.31	0.00000		0.00000	+					0.00000					8	1732.79	-3449.40	79.68	0.00
8	0.30	0.00001		-0.00004											4	1723.74	-3439.40	89.63	0.00
23	0.30	0.00000		-0.00003						0.00000					5	1723.83	-3437.60	91.46	0.00

Table S3.9 – Results from model selection for Cranial box; Model = Model number; (Int) = intersection; Elv = Elevation; Ltt = Latitude; Lng = Longitude; Ssn = Season; df = degrees of freedom; logLik = log likelihood; AICc = second-order Akaike information criterion; delta = difference between a model and the highest scoring mode; weight= model weight, the relative likelihood of a model ;"+" = factor incorporated into the model.

Model	(Int)	Elv	Ltt	Lng	Ssn	Sex	Elv:Ssn	Lng:Ssn	Ltt:Ssn	Elv:Lng	Ltt:Lng	Elv:Ltt	Elv:Sex	Ltt:Sex	Lng:Sex	df	logLik	AICc	delta	weight
29	-3723.0	1.04	310.10	-256.30		+				0.01901	3.81800	0.02352				9	-5551.39	11121.10	0.00	0.37
26	-2686.0	0.93	293.20	-240.30	+	+				0.01692	3.57100	0.02266				12	-5548.38	11121.30	0.20	0.34
46	-3192.0	1.47	311.50	-234.30	+	+				0.01288	3.52000					11	-5550.79	11124.00	2.92	0.09
42	858.6	-0.66	232.90	-207.90	+	+					3.03500	0.01711				11	-5550.85	11124.10	3.04	0.08
47	-4247.0	1.60	329.30	-250.40		+				0.01483	3.76900					8	-5553.97	11124.20	3.09	0.08
43	137.1	-0.76	244.60	-221.50		+					3.24400	0.01724				8	-5554.51	11125.30	4.18	0.05
28	1037.0	-0.70	229.80	-208.40	+	+			+		3.05600	0.01796				14	-5550.47	11129.60	8.55	0.01
31	-4396.0	1.28	329.60	-262.90						0.02046	3.94600	0.02141				8	-5562.90	11142.00	20.96	0.00
30	-3802.0	1.22	321.70	-250.40	+					0.01877	3.77200	0.02055				11	-5560.24	11142.90	21.83	0.00
49	-4861.0	1.78	346.80	-257.50						0.01662	3.90000					7	-5564.96	11144.10	23.03	0.00
48	-4243.0	1.71	337.80	-244.80	+					0.01506	3.72200					10	-5562.15	11144.60	23.57	0.00
21	-1039.0		279.00	-222.50	+						3.32200					8	-5564.36	11145.00	23.88	0.00
24	-1046.0		280.40	-224.30							3.34700					5	-5567.43	11145.00	23.88	0.00
15	-787.3	0.08	277.00	-215.70	+						3.23100					9	-5564.14	11146.60	25.49	0.00
44	111.9	-0.54	255.40	-214.60	+						3.18100	0.01432				10	-5563.16	11146.70	25.60	0.00
19	-1151.0	-0.03	281.40	-226.70							3.38100					6	-5567.40	11146.90	25.86	0.00
45	-248.8	-0.66	259.40	-225.60							3.33100	0.01460				7	-5566.39	11147.00	25.89	0.00
38	17340.0	-1.59	-91.07	-48.62	+	+				-0.00965		0.01920				11	-5573.25	11168.90	47.85	0.00
34	15270.0	0.29	-65.95	-56.95	+	+									+	10	-5574.48	11169.30	48.24	0.00
1	15920.0	0.32	-62.52	-48.97	+	+										9	-5576.65	11171.60	50.51	0.00
32	15860.0	0.39	-63.13	-48.98	+	+							+			10	-5575.86	11172.10	51.00	0.00
33	15470.0	0.32	-52.66	-49.02	+	+								+		10	-5575.95	11172.30	51.18	0.00
37	15640.0	0.21	-77.98	-61.21		+									+	7	-5582.00	11178.20	57.10	0.00
27	15070.0	0.39	-48.85	-50.35	+	+	+		+							15	-5573.71	11178.20	57.11	0.00
39	17790.0	-1.68	-103.50	-51.90		+				-0.00952		0.01991				8	-5581.26	11178.70	57.67	0.00
4	16360.0	0.24	-74.11	-52.17		+										6	-5584.70	11181.50	60.46	0.00
36	15830.0	0.24	-62.37	-52.24		+								+		7	-5583.74	11181.70	60.58	0.00
35	16300.0	0.31	-74.67	-52.18		+							+			7	-5584.01	11182.20	61.13	0.00
2	14060.0	0.68		-34.99	+	+										8	-5585.06	11186.30	65.26	0.00
16	16970.0	-0.55	-81.41	-50.48	+							0.02021				9	-5587.92	11194.10	73.05	0.00
40	17360.0	-1.42	-83.28	-47.84	+					-0.00925		0.01660				10	-5587.07	11194.50	73.41	0.00
14	16770.0	-1.00	-65.90	-45.45	+					-0.01195						9	-5588.21	11194.70	73.64	0.00
3	16070.0	0.33	-58.10	-48.37	+											8	-5589.73	11195.70	74.61	0.00
13	15680.0	0.33	-51.76	-49.47	+				+							11	-5587.80	11198.00	76.94	0.00
12	16020.0	0.34	-56.15	-47.85	+			+								11	-5589.13	11200.70	79.61	0.00
18	17470.0	-0.68	-95.33	-54.14								0.02097				6	-5594.51	11201.10	80.07	0.00
11	16020.0	0.37	-57.96	-48.17	+		+									11	-5589.44	11201.30	80.23	0.00
41	17850.0	-1.52	-97.37	-51.65						-0.00898		0.01747				7	-5593.72	11201.60	80.54	0.00
17	17250.0	-1.08	-79.23	-49.22						-0.01183						6	-5594.97	11202.10	81.00	0.00

5	16550.0	0.23	-71.23	-52.04										5	-5596.43	11202.90	81.87	0.00
9	17280.0		-96.74	-58.90										4	-5598.96	11206.00	84.90	0.00
7	14330.0	0.66		-35.36	+									7	-5596.73	11207.60	86.57	0.00
20	14490.0	0.22		-33.77	+				-0.00410					8	-5596.54	11209.30	88.24	0.00
8	14450.0	0.62		-36.59										4	-5607.87	11223.80	102.73	0.00
23	14490.0	0.50		-36.18					-0.00108					5	-5607.86	11225.80	104.74	0.00
6	14960.0	1.10	51.08		+									7	-5635.71	11285.60	164.52	0.00
22	14800.0	1.25	54.48		+						-0.00371			8	-5635.65	11287.50	166.46	0.00
10	15920.0	0.99	40.94											4	-5652.91	11313.90	192.81	0.00
25	15670.0	1.24	46.60								-0.00606			5	-5652.77	11315.60	194.56	0.00

CHAPTER 4

Gene expression responses of the American red squirrel (*Tamiasciurus hudsonicus*) to temperature and elevation

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ABSTRACT

Animals are faced with varied environmental conditions throughout their distribution. Many of these will put severe strains on their physiology and often species limits are determined by physiological constraints. Populations of mammals faced with extreme temperatures may show physiological responses reflecting adaptive evolutionary changes or there may be plastic responses mediated through changes in gene expression.

The focus of our work was the American red squirrel *Tamiasciurus hudsonicus*, a small widespread North American boreal forest mammal. *T.hudsonicus* does not hibernate or go into torpor and over its species range is exposed to widely varying climatic conditions, when considering seasons, elevation and latitude. Here we examine changes in gene expression relating to geographical and seasonal differences using RNAseq. We interpreted these in terms of broad gene ontology (GO) categories. We found upregulation in genes relating to oxidative metabolism and lipid metabolic reactions in populations found in cold or high elevation areas although this trend was mainly due to pronounced effects at high elevation. We found

upregulation relating to mitochondrial function in all warm or low elevation population and upregulation relating to iron binding in summer samples from New York compared to winter samples from the same population.

Overall, from the comparisons among *T.hudsonicus* that we were able to make, those that involved exposure to a combination of different elevation and different temperature showed greater differential gene expression than those comparisons that only involve exposure to a different temperature but not a different elevation. However, for the summer versus winter comparison, the differential gene expression suggests reduced metabolic activity in the winter.

INTRODUCTION

Adaptation can take on many forms including evolutionary change or plastic responses, and this can be illustrated by adaptation to different thermal conditions, of interest here. Thermal adaptations can include changes in gross morphology such as reducing size to increase heat exchange with the environment in warm areas (Shrestha et al 2014; Yom-Tov 1986) or changes in DNA sequences leading to, for example, structural changes in proteins allowing for better transfer of oxygen to enhance metabolism at cold temperatures. Adaptive plastic responses relating to thermal conditions include behavioral changes such as individuals maximizing foraging time during dusk and dawn under high temperatures to avoid being out during the hottest time of day when they would be out of the thermoneutral zone (Cain et al 2006), or changes in gene expression e.g. upregulation of uncoupling proteins in brown adipose tissue to generate heat through non-shivering thermogenesis during cold conditions (Nedergaard et al 2001).

One aspect of thermal adaptation is coping with extreme conditions. Even if temperatures are tolerable for much of the time (perhaps directed behavior may contribute to the maintenance

of such generally tolerable conditions), individuals may be exposed to extreme temperatures for a period, leading to cold or heat stress. An individual in cold or heat stress is outside its thermal neutral zone and having to expend energy to maintain an appropriate constant core body temperature (Feldhamer et al 2016). There are various ways in which endotherms can deal with cold and heat stress through physiological changes. When individuals suffer cold stress heat production needs to be increased to compensate increased heat loss in order to maintain constant body temperature (Venditti et al 2016). This heat production can take the form of shivering, in muscles, or non-shivering, in brown adipose tissue. When individuals face heat stress they may increase evaporative cooling (such as sweating or panting) and increase blood flow close to the skin (Huey et al 2012). Many of the metabolic changes observed in one tissue, such as the non-shivering thermogenesis response in brown adipose tissue, can also be detected in other tissues such as liver, skeletal muscle and kidney (Guernsey & Stevens 1977; Shore et al 2013). These physiological changes are likely to be associated with changes in gene expression. There is a large literature on patterns of up- and down-regulation of particular genes that are associated with hot or cold stress (Sonna et al 2002; Fujita 1999; Lindquist 1986). Examples include the heat response of down-regulation of gene transcripts associated with protein synthesis and cellular metabolism in response to morphological changes and reduced cell growth, upregulation of genes associated with DNA and protein repair and short term elevation of expression of HSP70 before ultimate cell death, as recorded in bovine mammary epithelial cells (Collier et al 2005; Collier et al 2008). An example of cold response is the upregulation of UCP1 (or Themogenin) in brown adipose tissue which increases permeability of the inner mitochondrial membrane thereby increasing heat dissipation and lowering ATP production (Cannon & Nedergaard 2004)

To understand thermal tolerance in terms of evolutionary adaptation and thermal plastic responses it is important to know how species cope with a wide range of temperatures over their natural geographical range. It is particularly instructive to examine species with large geographical ranges that incorporate different thermal conditions. Under those circumstances it may be possible to examine a single species that is subject to hot and cold stresses in different parts of their distribution at different times of year. This reflects systematic differences in the thermal regime that may have influence on the adaptive response.

The American red squirrel, *Tamiasciurus hudsonicus*, a small highly active mammal, is exceptional in this respect. It has an extremely wide distribution in North America, from Alaska in the northwest, through most of Canada to the eastern seaboard and following the coniferous and mixed coniferous forest along the Rocky Mountains in the west and Appalachians in the east (Steele 1998). The species does not hibernate nor go into daily torpor (Brigham & Geiser 2011). It is therefore active all year round over a very large geographical range, with limited behavioral protection (it needs to forage frequently, defend its territory etc. and therefore is exposed to the external environment for much of the time: Gurnell 1984; Pauls 1978). When examining the conditions throughout the species distribution it becomes apparent that *T.hudsonicus* potentially has to be able to handle quite extreme thermal conditions.

In other chapters we investigate evolutionary adaptation of *T.hudsonicus* to extreme thermal conditions in terms of morphology. In this chapter, we investigate geographical and seasonal differences in gene expression using RNAseq (Nagalakshmi et al 2008; Wang, Gerstein & Snyder 2009). This could be due to constitutive changes in gene expression with a genetic basis; therefore evolutionary adaptation. It could also be a plastic response, in which an individual varies gene expression according to particular conditions.

This study is preliminary in various respects. It is restricted to a single organ type (liver), to a small number of comparisons with low replication. However, we focus on genes that are upregulated in multiple comparisons comparing warmer and colder conditions and *vice versa*, and through gene ontology we interpret the function of these genes. Although it does not provide the complete picture of gene expression differences between *T.hudsonicus* in different thermal settings, liver is a particularly appropriate organ to study. Shore et al (2013) note, for instance, that the metabolic response to cold in mammals critically involves liver (e.g. through gluconeogenesis), and it is also an organ of choice in analyzing aspects of heat stress (e.g. for the detection of heat shock proteins Salway et al 2011). In short, liver is an exceptionally important mammalian organ for studying metabolic processes (Rui 2014).

MATERIALS AND METHODS

Sample collection

Red squirrels were collected between January 2013 and June 2014 in New York, West Virginia, Colorado, Montana and Alaska (table 4.1) with appropriate permitting and following an approved Cornell IACUC protocol. Immediately after euthanasia individuals were dissected using sterilized instrument and liver tissues collected. Tissue samples were placed directly into RNAlater (Invitrogen) in the field and placed and stored at -80C until RNA was extracted. Only males were used in this analysis.

Table 4.1 – Samples (collected in the summer unless otherwise stated) used in the study with location data and group allocation (NYw and NYs being winter and summer samples from New York State)

Sample	Group	Location	Latitude	Longitude	Elevation (m)	Date
Th024	NYw	Adirondacks	44.249031	-73.985689	550	30 Jan '13
Th025	NYw	Adirondacks	44.249031	-73.985689	550	31 Jan '13
Th069	NYs	Adirondacks	44.249031	-73.985689	550	09 Jul '13
Th079	NYs	Adirondacks	44.249031	-73.985689	550	29 Jul '13
Th031	MT	Lubrecht Forest	46.8937	-113.44779	1256	16 Jun '13
Th034	MT	Lubrecht Forest	46.8937	-113.44779	1256	17 Jun '13
Th050	COs	West Elk	38.79333	-107.245556	2744	29 Jun '13

Th052	COs	West Elk	38.79333	-107.245556	2744	29 Jun '13
Th090	COw	West Elk	38.79333	-107.245556	2744	13 Feb '14
Th091	COw	West Elk	38.79333	-107.245556	2744	13 Feb '14
Th071	WV	Cheat Mountain	38.629051	-79.925799	626	22 Jul '13
Th073	WV	Cheat Mountain	38.629051	-79.925799	626	22 Jul '13
Th120	AK	Tanana State Forest	63.214167	-143.06555	550	21 Jun '14
Th122	AK	Tanana State Forest	63.214167	-143.06555	550	21 Jun '14

Molecular work

Total RNA was extracted using an RNAdvance tissue kit (Beckman Coulter). RNA was quantified and integrity assessed using a Bioanalyzer 2100.

Libraries were generated using the Illumina TruSeq RNA sample preparation kit v2 following the low sample protocol according to the manufacturer's instructions. RNA (1 µg) was subjected to poly-A messenger RNA (mRNA) selection using oligo-dT attached magnetic beads followed by chemical fragmentation (8 min, 94 °C). Cleaved RNA fragments were then copied into first strand cDNA using SuperScript II reverse transcriptase (Invitrogen) and Illumina proprietary random hexamer primers. After second strand synthesis using Illumina-supplied consumables, purification of the cDNA with Agencourt AMPure XP beads (Beckman Coulter), and overhang end repair with adenylation of the 3' blunt ends, the cDNA was ligated to barcoded adapters. The barcoded cDNA was then enriched for fragments that have adapter molecules on both ends and amplified with PCR reagents of the same kit. The final libraries were amplified using 14 PCR cycles. We quantified and assessed library quality on an Advanced Analytical Fragment Analyzer using Standard Sensitivity NGS Fragment Analysis.

Following the standard quality filtering by Illumina we estimated the read quality using FastQC version 0.11.4 (Andrews 2010). To ensure the quality of downstream analysis Trimmomatic 0.36 (Bolger, Lohse & Usadel 2014) was used to trim the first 15 bp of each read, trim the 5' and/or 3' end of reads where the quality score went below Q20, trim off anywhere

within a read where the quality of a 5bp window was below Q20, and remove any reads less than 36bp long.

We used multidimensional scaling plots to visualize the genetic structure of the data and following that excluded sample Th091 based on that result and due to low number of reads.

Gene expression analysis

Reads were aligned to an annotated thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) transcriptome using TopHat 2.1.1 (Trapnell et al 2012). A range of 14.3-43.3% of reads from different samples mapped to the transcriptome. Mapped reads were sorted and indexed using SAMtools (Li & Durbin 2009). We analyzed differential gene expression and carried out TMM normalization (Robinson & Oshlack 2010) using the edgeR (Robinson, McCarthy & Smyth 2010) R package (R version 3.2.5, R Development Core Team). We considered genes with more than two fold change and a false discovery rate corrected *P* value of less than 0.05 to be differentially expressed. We discarded all genes where there were fewer than 20 reads and when more than 50% of the samples had no reads. We tested differential gene expression for a number of comparisons (table 4.2). To reduce bias in comparisons due to unequal sample sizes we excluded the second Colorado winter sample (Th090) at this stage.

It is worth noting that when denoting upregulation we are simply comparing the two populations in question and calling the genes upregulated when more reads are found in one population. This is done for the sake of convenience but another explanation for the difference could also be that the genes in the second population are being downregulated.

Table 4.2 – Group comparisons made for gene expression differences, with groups and samples defined as in table 4.1

Comparison	Samples being tested	Variables being tested
NYs vs NYw	Th069 & Th079 vs Th024 & Th025	Temperature – All samples come from the same location and same population but collected in the warmest vs coldest months of the year.
NY vs WV	Th069 & Th079 vs Th071 & Th073	Temperature – Elevation is nearly identical while New York is further north than West Virginia and therefore lower temperature.
NY vs AK	Th069 & Th079 vs Th120 & Th122	Temperature – The locations are different latitude and therefore New York has higher temperatures in the summer. Elevation is similar.
WV vs CO	Th071 & Th073 vs Th050 vs Th052	Elevation and temperature – While both locations are at similar latitude Colorado is at a much higher elevation and therefore also colder.
NY vs MT	Th069 & Th079 vs Th031 & Th034	Elevation and temperature – The locations are at a similar latitude but Montana is at higher elevation and therefore lower temperature.

In total we made five comparisons in order to identify gene expression differences that may be related to either thermal or elevational tolerance (table 4.2). It should be noted that the New York summer samples were used in all comparisons except for one (WV vs CO). Our sample sizes are small and in order to be even more stringent in our selection of upregulated genes, we selected genes which were upregulated in more than one geographical comparison for particular scrutiny; we call these 'comparative comparisons'. Even more than that, we particularly focus on genes that appear in more than one comparative comparison. We also include the New York summer vs. New York winter comparison as an additional contrast in these analyses. Gene ontology (GO) terms and descriptions of gene activities were acquired using the Gene Ontology Consortium resources (Ashburner et al 2000; www.geneontology.org) and Genecards (www.genecards.org). For our GO term choices we followed Shore et al's (2013) study on cold exposure in mice plus additional terms important in metabolism.

RESULTS

The highest number of differentially expressed genes was found between West Virginia and

Colorado and fewest in the comparison between seasons in New York (table 4.3).

Table 4.3 – Number of differentially expressed genes in comparisons described in table 4.2

Comparison	Upregulated in 1	Upregulated in 2	Number of differentially expressed genes
NYs vs NYw	36	20	56
NY vs AK	90	104	194
NY vs WV	31	32	63
NY vs MT	115	123	238
WV vs CO	252	338	590

Over the 20 comparative comparisons, there were plentiful examples of genes that were

upregulated in both comparisons ('co-upregulated')(table 4.4). Considering co-upregulation in

cold or high elevation conditions, the highest number of co-upregulated genes were found in the

high elevation populations in Colorado and Montana when compared to low elevation

populations at similar latitudes. Similarly, considering co-upregulation in warm or low elevation

conditions, a large number of genes were found to be co-upregulated in the low elevation

populations compared to the high elevation populations. No co-upregulated genes were found in

comparisons involving the three populations at the same elevation (New York, Alaska and West

Virginia), although there were upregulations in common between Alaska (vs New York) and

New York winter (vs New York summer) and between New York (vs Alaska) and New York

summer (vs New York winter).

Table 4.4 – Co-upregulation of genes in comparative comparisons (see text)

Comparative comparisons 1	2	Upregulated in 1	Upregulated in both	Upregulated in 2
Cold or high elevation				
CO(vsWV)	MT(vsNY)	307	31	92
NY(vsWV)	CO(vsWV)	10	21	317
AK(vsNY)	CO(vsWV)	92	12	326
AK(vsNY)	MT(vsNY)	97	7	116
AK(vsNY)	NYw(vsNYs)	13	7	97
MT(vsNY)	NYw(vsNYs)	117	6	14
CO(vsWV)	NYw(vsNYs)	337	1	19
NY(vsWV)	MT(vsNY)	30	1	122
NY(vsWV)	NYw(vNYs)	31	0	20
NY(vsWV)	AK(vsNY)	31	0	104
Warm or low elevation				
NY(vsMT)	WV(vsCO)	92	23	229
WV(vsNY)	WV(vsCO)	10	22	230
NY(vaAK)	NY(vsMT)	71	19	96
NY(vsAK)	WV(vsCO)	77	13	239
NY(vsMT)	NYs(vsNYw)	107	8	28
WV(vsCO)	NYs(vsNYw)	245	7	29
NY(vsAK)	NYs(vsNYw)	84	6	30
WV(vsNY)	NY(vsMT)	31	1	114
WV(vsNY)	NYs(vsNYw)	32	0	36
WV(vsNY)	NY(vsAK)	32	0	90
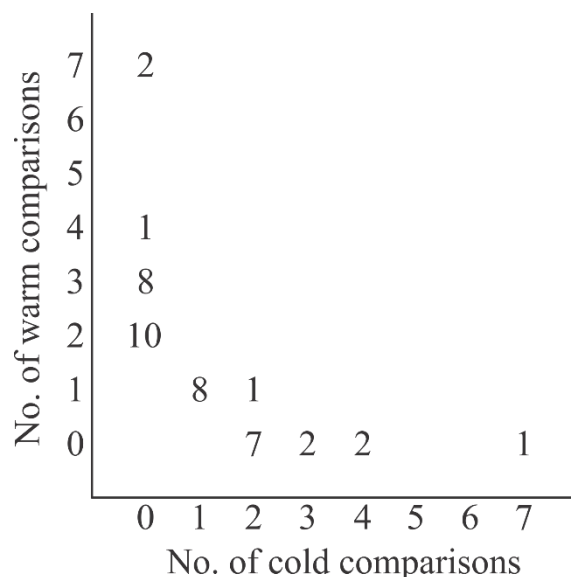
Figure 4.1 - Number of genes showing upregulation for different numerical combinations of cold and warm comparative comparisons.

Various genes were found to share upregulation between several of the comparative comparisons from table 4.4 and are listed in table 4.5 together with examples of sharing with the New York summer vs. New York winter comparison. In almost all cases the shared upregulation was found within either the cold/high elevation category or the warm/low elevation category; this is particularly true when there are three or more shared comparisons (figure 4.1). The most commonly

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upregulated gene in the winter (ENSSTOG00000011815) was not found annotated in the thirteen-lined ground squirrel and no results were found using BLAST (Altschul et al 1990; http://blast.ncbi.nlm.nih.gov/Blast.cgi). This gene was found upregulated in both the high elevation sites (MT and CO) compared to their latitudinal counterparts (NY and WV), found in many cold vs hot comparisons (such as AK(vsNY) and NYw(vsNY)) and in comparisons grouping high elevation (CO(vsWV) and low temperatures (NYw(NYs)). Two genes (CYP7B1, CYP26B1), members of the cytochrome P450 oxidoreductase group of enzymes were found upregulated in three and two cold or high elevation comparisons, respectively. The most commonly shared upregulated gene in warm or low elevation sites (ENSSTOG00000028432) relates to metabolic activity and was found upregulated in all comparisons apart from those involving upregulation in WV vs NY.

Table 4.5 – Differentially expressed genes upregulated in more than one of the comparative comparisons from table 4.4 in addition to the comparison between New York winter and New York summer

Gene name	Symbol	Description	Comparison*
ENSSTOG00000011815	NA	NA	●●●●●●●
ENSSTOG00000020259	NA	Carbohydrate metabolic process	●●●●
ENSSTOG00000024583	CISH	Degradation of this protein in involved in inactivation of erythropoietin receptors	●●●●
ENSSTOG00000024724	SLC16A11	Involved in hepatic lipid metabolism	●●●
ENSSTOG00000010923	CYP7B1	Member of the cytochrome P450 group. Endoplasmic reticulum protein important in cholesterol catabolic pathway converting cholesterol to bile	●●●

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ENSSTOG00000006538	ATOH8	Transcription factor that binds an E-box element	
ENSSTOG00000021006	FGF21	Stimulates the uptake of glucose in adipose tissue	
ENSSTOG00000007640	ID1	Inhibitor of DNA binding, may play a role in cell growth, senescence and differentiation.	
ENSSTOG00000019401	ID2	Inhibitor of DNA binding, may play a role in negatively regulating cell differentiation.	
ENSSTOG00000024534	NA	NA	
ENSSTOG00000002629	KDELR3	Endoplasmic reticulum protein, required for normal vesicular traffic through the Golgi.	
ENSSTOG00000021197	NA	NA	
ENSSTOG00000021898	CYP26B1	Member of the cytochrome P450 group. Found in the endoplasmic reticulum, a regulator of all-trans retinoic acid levels	
ENSSTOG00000028432	NA	Involved with metabolic activity in relation to the mitochondrion	
ENSSTOG00000001482	RET	Involved in numerous processes including cell proliferation, migration and differentiation.	
ENSSTOG00000004459	NA	Component of membrane	
ENSSTOG00000004982	TRIM63	Regulates degradation of muscle proteins under amino acid starvation	
ENSSTOG00000008679	CRISPLD1	NA	
ENSSTOG00000020350	EIF4EBP3	Repressor of translation initiation that regulates EIF4E	
ENSSTOG00000021636	PPP1R3G	Involved in the regulation of hepatic glycogenesis coupled to the fasting-feeding cycle	

ENSSTOG00000022426	TSPAN32	Tumor suppressing gene, a member of the tetraspanin superfamily	
ENSSTOG00000022668	EVPL	Component of the cornified envelope of keratinocytes	
ENSSTOG00000026217	SACS	Regulator of the Hsp70 chaperone machinery, may regulate other ataxia proteins.	
ENSSTOG00000027573	COMT	Involved in the metabolic degradation of catecholamine neurotransmitters and catechol hormones	
ENSSTOG00000005711	IRF7	Plays a critical role in the innate immune response against viruses	
ENSSTOG00000007829	TNNI2	Responsible for calcium-dependent regulation of striated muscle concentration.	
ENSSTOG00000028109	CPNE6	Calcium dependent, phospholipid-binding, may play a role in membrane trafficking	
ENSSTOG00000007820	NA	Integral component of Golgi membrane, involved in transferase activity	
ENSSTOG00000025236	CFAP100	Cilia and flagella associated protein	
ENSSTOG00000001180	CD163	Expressed in monocytes and macrophages. May play a role in the uptake and recycling of iron	
ENSSTOG00000026716	VCAN	Plays a central role in tissue morphogenesis and maintenance	
ENSSTOG00000020288	ISG15	Ubiquitin-like protein which plays a key role in the innate immune response to viral function	
ENSSTOG00000006404	PTCH2	May function as a tumor suppressor and as a receptor for Sonic hedgehog	
ENSSTOG00000009418	PLCD3	Activated by calcium induces the creation diacylglycerol and IP3	
ENSSTOG00000003382	TNC	Relating to neural pathways	
ENSSTOG00000028862	EEF1A2	Translation elongation factor essential in protein synthesis	
ENSSTOG00000003590	ROPN1L	Only described function in sperm tails	
ENSSTOG00000027370	GADD45G	Responds to environmental stresses	
ENSSTOG00000011601		Involved in sodium ion transport across membranes	
ENSSTOG00000024424	NA	Involved with exocytosis	
ENSSTOG00000003422	CAPN9	Calcium binding. Implicated in neurodegenerative processes as their activation can be triggered by calcium influx and oxidative stress	

| ENSSTOG00000000900 | SERPINA6 | Transport protein for glucocorticoids and progestins in the blood | ○ ● |

*Color codes for comparisons

- ● NY(vsWV) & CO(vsWV)
- ● NY(vsWV) & MT(vsNY)
- ● AK(vsNY) & CO(vsWV)
- ● CO(vsWV) & NYw(vsNYs)
- ● AK(vsNY) & MT(vsNY)
- ● AK(vsNY) & NYw(vsNYs)
- ● MT(vsNY) & CO(vsWV)
- ● MT(vsNY) & NYw(vsNYs)
- ● NYw(vsNYs)

- ● WV(vsNY) & WV(vsCO)
- ● WV(vsNY) & NY(vsMT)
- ● NY(vsAK) & WV(vsCO)
- ● WV(vsCO) & NYs(vsNYw)
- ● NY(vsAK) & NY(vsMT)
- ● NY(vsAK) & NYs(vsNYw)
- ● NY(vsMT) & WV(vsCO)
- ● NY(vsMT) & NYs(vsNYw)
- ○ NYs(vsNYw)

Data relating to frequently represented GO-terms are summarized in table 4.6. More genes and hits were found associated with the GO terms Oxidoreductase activity and Endoplasmic reticulum membrane when there is co-upregulation in cold or high elevation populations while the GO term Mitochondrion was more commonly encountered when there was co-upregulation in warmer or lower elevation populations (table 4.6; tables S4.1- S4.18). All four genes associated with Iron ion binding in warm or low elevation populations were found upregulated in New York summer compared to New York winter. For cold or high elevation populations co-upregulated genes associated with Iron ion binding always also showed Oxidoreductase activity. No co-upregulated genes in warm or low elevation populations were associated with more than one of the target GO terms. The GO term Mitochondrion was associated with at least one co-upregulated genes in all of the warm or low elevation comparative comparisons in table 4.4. In contrast, Mitochondrion was only associated with two co-upregulated genes over all the cold or high elevation comparative comparisons in table 4.4 in one case associated with Oxidoreductase activity (in the CO(vsWV) & MT(vsNY) comparison) and in the other associated with Carbohydrate metabolic processes (in the CO(vsWV) & NY(vsWV) comparison). Five of the hits for Oxidoreductase activity seen in the cold or high elevation comparative comparisons was in the comparison CO(vsWV) & MT(vsNY), four of those hits were due to genes that were only upregulated in this comparison and three of those were also

associated with Lipid metabolic processes. Twelve out of 38 total hits in cold or high elevation

populations were due to the CO(vsWV) & MT(vsNY) comparative comparison. For the warm or

low elevation populations the NYs(vsNYw) accounted for nine out of 30 hits.

Table 4.6 – Frequently represented GO-terms in differentially expressed genes, co-upregulated in comparative comparisons (table 4.4), identifying numbers of genes involved and also number of hits which includes examples where the same gene is involved in multiple comparisons

GO Term	Upregulated in cold or high elevation populations		Upregulated in warm or low elevation populations	
	Number of genes	Number of hits	Number of genes	Number of hits
Endoplasmic reticulum membrane	3	8	0	0
Iron ion binding	2	5	4	4
Oxidoreductase activity	8	11	0	0
Carbohydrate metabolic process	2	5	3	3
Lipid metabolic process	5	7	3	4
Mitochondrion	2	2	8	19

DISCUSSION

There are reasons for caution with our study given low sample sizes (requiring reuse of some

samples for different comparisons) and the fact that we have not been able to support our data

from field-collected specimens by controlled studies in the laboratory. However, despite these

limitations, our wide geographic comparisons in *T.hudsonicus* are particularly unusual,

interesting and informative in relationship to the role of temperature and elevation in differential

gene expression in wild organisms. Also, we have very good sequencing coverage for each

sample with a large number of reads aligning to the reference genome (mostly around 15 million

reads per sample), allowing us to make the very best of the samples that we do have for

analyzing gene expression.

    Based on the frequency of GO terms in upregulated genes, overall we can see an increase

in oxidative metabolism in cold or higher elevation populations. We see in these areas relatively

more upregulation in genes involved with lipid metabolic reactions, cholesterol and bile transport and metabolism. The comparative comparison that contributes most to this result is CO(vsWV) and MT(vsNY). However, there is also a strong tendency in the warm or low elevation populations for the upregulation of genes related to the mitochondrion.

In interpreting our results, while there is a common trend in active mammals of an increase in metabolic activity in cold conditions (e.g. Merritt 1986; Wang, Zhang & Wang 2006) it should not necessarily be assumed to be the case for this species. *T.hudsonicus* has been found to lower its body temperature in winter compared to summer, and outside of the nest compared to inside the nest (Pauls 1979). Additionally, in contrast to what has been found in many mammals, the field metabolic rate of *T.hudsonicus* is much lower in the winter than it is in the summer, indicating that the species is able to impressively minimize its energy expenditure during cold conditions (Humphries et al 2005; Hollerman, White & Feist 1982; Feist & White 1989). The species has been found to behaviorally reduce its energy requirements by basing nesting choices on thermal needs (individuals have multiple nests in their territory, they can be grass nests in trees, hollows in trees or burrows; Guillemette et al 2009) and by minimizing activity during winter compared to summer when activity is again reduced at temperatures above 20C (Pauls 1978; Ferron, Oullet & Lemay 1985).

That being said when we looked at comparative comparisons involving latitudinal differences but not elevational differences we did not see upregulation of shared genes. This could be due to the fact that while there is a considerable difference in the temperatures in these areas the northern location does not reach temperatures that would make it difficult to thermoregulate. Moreover, while temperature can be presumed to be higher further south, endotherms have been shown to alter their behavior to minimize time spent active during the

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hottest period of the day such as having a rest phase during midday (Váczi, Koósz & Altbäcker 2006). *T.hudsonicus* has been shown to have the most biphasic activity pattern during the summer (Layne 1954) but has additionally been seen to reduce its activity when temperatures go beyond 20C (Ferron, Oullet & Lemay 1985) indicating that behavior thermoregulation may be a factor in our results.

With this in mind two suggestions can be made to explain the data.

First of all it seems that metabolic responses to elevation are pronounced, although they may be confounded with temperature. The two comparisons showing the greatest number of differentially expressed genes are the NY vs MT and WV vs CO elevational comparisons. Additionally when we look at the comparative comparisons we find that most shared genes are found between MT(vsNY) and CO(vsWV) in the cold or high elevation populations and between WV(vsCO) and NY(vsMT) in the warm or low elevation populations. Species living at high elevation are faced with both lower temperatures than in nearby low elevation and reduced oxygen levels. If cold is a stressor endotherms will increase metabolic heat production to maintain a constant body temperature. This requires increased usage of oxygen due to usage of oxidative pathways. While work on humans has shown that acclimation to elevation is achieved through increased carbohydrate utilization (Marconi, Marzorati & Cerretelli 2006; Brooks et al 1991) perhaps to enhance O_2 economy rather than maximize aerobic capacity (Storz, Scott & Cheviron 2010), research on deer mice (*Peromyscus maniculatus*) has shown a shift towards the use of lipids instead of carbohydrates as fuels (Cheviron et al 2012). Our findings suggest that *T.hudsonicus* may have similar adaptive responses to elevational acclimation as *P.maniculatus*. We found the most upregulation in genes involved with oxidoreductase and lipid metabolism suggesting that the species is maximizing aerobic capacity rather than just O_2 economy.

Secondly, the increase in mitochondrial activity observed in all warm or low elevation comparisons could be due to the increase activity of *T.hudsonicus* in the summer months compared to the winter months. It might additionally be that rather than upregulation of these genes in warm and low elevation populations there is downregulation in cold and high elevation populations. If that is the case we might be seeing indicators of the lowered activity, reduced body temperature and lowered field metabolic rate in colder environments. Moreover, genes relating to iron binding are differentially expressed in winter vs summer in NY, again upregulated in summer (or downregulated in winter). Given the importance of iron ion oxygen transport this again could be an indicator of difference in metabolic activity between cold and warm environments.

Overall our data supports adaptive response to elevation based on shift to lipid metabolism in response to oxidative stress and hints at a possible change in metabolic activity between colder and warmer areas/seasons.

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SUPPLEMENTARY MATERIAL 4

Table S4.1 - Over expressed genes in both AK and MT (NYvsMT and NYvsAK).

Gene	Name	Description	Gene cards	GO Terms
ENSSTOG00000011815	Missing	Missing		▪ Calcium ion binding ▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules cell adhesion ▪ Extracellular exosome ▪ Cytoplasm ▪ Corticospinal tract morphogenesis
ENSSTOG00000015287	CXCL13	C-X-C motif chemokine ligand 13	B lymphocyte chemoattractant. May function in the homing of B lymphocytes to follicles. Diseases associated with the gene include angoioimmunoblastic t-cell lymphoma and burkitt lymphoma	▪ Chemokine activity ▪ Immune response ▪ Chemotaxis ▪ Cytokine activity ▪ Extracellular space ▪ Extracellular region ▪ Defense response to bacterium ▪ Protein heterodimerization activity ▪ Cell surface receptor signaling pathway ▪ Cell-cell signaling ▪ B cell chemotaxis ▪ Positive regulation of cytosolic calcium ion concentration ▪ Heparin binding ▪ Receptor agonist activity ▪ CXCR3 chemokine receptor binding ▪ Positive regulation of T cell chemotaxis ▪ CCR10 chemokine receptor binding

				- Fibroblast growth factor binding - CXCR5 chemokine receptor binding - Endothelial cell chemotaxis to fibroblast growth factor - Negative regulation of endothelial cell chemotaxis to fibroblast growth factor - Activation of GTPase activity - Positive regulation of integrin activation - Positive regulation of cell-cell adhesion mediated by integrin - Lymph node development - Lymphocyte chemotaxis across high endothelial venule - B cell chemotaxis across high endothelial venule - Cell chemotaxis
ENSSTOG00000020259	Missing	Missing		- Hydrolase activity, hydrolyzing O-glycosyl compounds - Carbohydrate metabolic process
ENSSTOG00000021006	FGF21	fibroblast growth factor 21	Member of the fibroblast growth factor family. FGF family members possess broad mitogenic and cell survival activities. This protein is a secreted endocrine factor that functions as a major metabolic regulator. The encoded protein stimulates the uptake of glucose in adipose tissue.	- Fibroblast growth factor receptor signaling pathway - Growth factor activity - Fibroblast growth factor receptor binding - Receptor binding - Extracellular region - Protein binding - Positive regulation of ERK1 and ERK2 cascade - Positive regulation of glucose import - Positive regulation of cell proliferation - Positive regulation of MAPKKK cascade by fibroblast growth factor receptor signaling pathway

ENSSTOG00000022144	ARNTL	aryl hydrocarbon receptor nuclear translocator like	The protein encoded by this gene is a basic helix-loop-helix protein that forms a heterodimer with CLOCK. This heterodimer binds E-box enhancer elements upstreams of Period (PER1, PER2, PER3) and Cryptochrome (CRY1, CRY2) genes and activates transcription of these genes. Defects in this gene have been linked to infertility, proglems with gluconeogenesis and lipogenesis, and altered sleep patterns. Among its related pathways are metabolism and regulation of lipid metabolism by Peroxisome proliferation-activated receptor alpha (PPARalpha).	▪ DNA binding ▪ Transcription factor complex ▪ Transcription from RNA polymerase II promoter ▪ Transcription factor activity, sequence-specific DNA binding ▪ Transcription, DNA-templated ▪ Cytoplasm ▪ Protein dimerization activity ▪ Regulation of transcription, DNA-templated ▪ Nucleus ▪ Protein binding ▪ Positive regulation of transcription from RNA polymerase II promoter ▪ Positive regulation of transcription, DNA-templated ▪ Nucleoplasm ▪ Intracellular membrane-bounded organelle ▪ Aryl hydrocarbon receptor binding ▪ Circadian regulation of gene expression ▪ Regulation of hair cycle ▪ Response to redox state ▪ Hsp90 protein binding ▪ Repressing transcription factor binding ▪ E-box binding
ENSSTOG00000024583	CISH	cytokine inducible SH2 containing protein	The expression of this gene can be induced by IL2, IL3, GM-CSF and EPO in hematopoietic cells . Proteasome-mediated degradation of this protein has been shown to be	▪ Intracellular ▪ Protein ubiquitination ▪ Intracellular signal transduction ▪ Protein binding ▪ Plasma membrane ▪ Protein kinase C-activating G-protein coupled receptor signaling pathway

			involved in the inactivation of the erythropoietin receptors.	
ENSSTOG00000024724	SLC16A11	solute carrier family 16 member 11	Probably involved in hepatic lipid metabolism: overexpression results in an increase of triacylglycerol (TAG) levels, small increase in intracellular diacylglycerols and decreases in lysophosphatidycholine, cholesterol ester and sphingomyelin lipids.	▪ Integral component of membrane ▪ Membrane ▪ Transmembrane transport ▪ Endoplasmic reticulum membrane ▪ Lipid metabolic process

Table S4.2 - Over expressed genes in both CO and MT (NYvsMT and COvsWV).

Gene	Name	Description	Gene cards	GO Terms
ENSSTOG00000002703	MYO7A	myosin VIIA	This gene encodes an unconventional myosin with a very short tail, defect causes Usher syndrome (hearing loss, night blindness and loss of peripheral vision).	- Myosin complex - ATP binding - Actin binding - Motor activity - Nucleotide binding - Cytoskeleton - Cytoplasm - Cytosol - Protein binding - Actin filament binding - Sensory perception of sound - Actin filament-based movement - Photoreceptor outer segment - Visual perception - Synapse - Calmodulin binding - Lysosomal membrane - Photoreceptor inner segment - Spectrin binding - Lysosome organization - Microfilament motor activity - Sensory perception of light stimulus - Equilibrioception - Intracellular protein transport - Protein domain specific binding - Stereocilium - Inner ear development - Inner ear morphogenesis - Apical plasma membrane - Phagolysosome assembly - Photoreceptor connecting cilium

				- Cell projection organization - Phagocytosis - Innear ear receptor stereocilium organization - Auditory receptor cell stereocilium organization - Melanosome - Sensory perception - Post-embryonic organ morphogenesis - Mechanoreceptor differentiation - Inner ear receptor cell differentiation - Auditory receptor cell differentiation - Pigment granule localization - Pigment granule transport
ENSSTOG00000003382	TNC	tenascin C	It is implicated in guidance of migrating neurons as well as axons during development, synaptic plasticity, and neuronal regeneration.	- Extracellular matrix - Cell adhesion - Regulation of cell proliferation - Extracellular space - Membrane - Focal adhesion - Response to wounding - Syndecan binding - Osteoblast differentiation - Extracellular region - Positive regulation of gene expression - Positive regulation of cell proliferation - Basement membrane - Neuromuscular junction development - Interstitital matrix - Prostate gland epithelium morphogenesis - Peripheral nervous system axon regeneration - Mesenchymal-epithelial cell signaling involved in prostate gland development

ENSSTOG00000003707	POLA2	polymerase (DNA) alpha 2, accessory subunit	May play an essential role at the early stages of chromosomal DNA replication by coupling the polymerase alpha/primase complex to the cellular replication machinery. Among its related pathways are Metabolism and Cell cycle.	▪ DNA binding ▪ DNA biosynthetic process ▪ DNA-directed DNA polymerase activity ▪ DNA replication ▪ Nucleus ▪ Cytoplasm ▪ Neucleoplasm ▪ Protein heterodimerization activity ▪ Protein binding ▪ Protein import into nucleus, translocation ▪ Alpha DNA polymerase: primase complex
ENSSTOG00000003970	Missing	Missing		▪ Extracellular exosome ▪ Cognition
ENSSTOG00000004186	EXOC3L4	exocyst complex component 3 like 4		▪ Exocyst ▪ Exocytosis
ENSSTOG00000004270	Missing	Missing		▪ Oxidation-reduction process ▪ Oxidoreductase activity ▪ Integral component of membrane ▪ Membrane ▪ Oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water ▪ Fatty acid biosynthetic process ▪ Lipid metabolic process
ENSSTOG00000004294	CCSER2	coiled-coil serine rich protein 2]		▪ Microtubule cytoskeleton ▪ Microtubule binding ▪ Microtubule bundle formation
ENSSTOG00000004984	Missing	Missing		▪ Intracellular ▪ Positive regulation of GTPase activity

				▪ Guanyl-nucleotide exchange factor activity ▪ Small GTPase mediated signal transduction
ENSSTOG00000005853	Missing	Missing		▪ Oxidation-reduction process ▪ Oxidoreductase activity ▪ Integral component of membrane ▪ Membrane ▪ Oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water ▪ Fatty acid biosynthetic process ▪ Lipid metabolic process
ENSSTOG00000007105	FNDC1	fibronectin type III domain containing 1		▪ Nucleoplasm
ENSSTOG00000008137	COL19A1	collagen type XIX alpha 1 chain	The function of this collagen are not known but others in the family are found in association with fibril-forming collagens such as type I and II, and serve to maintain the integrity of the extracellular matrix.	▪ Proteinaceous extracellular matrix ▪ Extracellular matrix organization ▪ Skeletal muscle tissue development
ENSSTOG00000008633	CADPS2	calcium dependent secretion activator 2	Calcium-binding protein involved in exocytosis of vesicles filled with neurotransmitters and neuropeptides. Probably acts upstream of fusion in the biogenesis or maintenance of mature secretory vesicles. Regulates neurotrophin	▪ Dense core granule exocytosis ▪ Synaptic vesicle exocytosis ▪ Cytosol ▪ Nucleoplasm ▪ Intracellular membrane-bounded organelle ▪ Protein binding ▪ Postsynaptic membrane ▪ Cytoplasmic, membrane-bounded vesicle ▪ Cytoplastic vesicle

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			release from granule cells leading to regulate cell differentiation and survival during cerebellar development.	- Presynaptic membrane - Cellular response to starvation - Positive regulation of exocytosis - Synaptic vesicle priming - Presynapse - Synapse
ENSSTOG00000010923	CYP7B1	cytochrome P450 family 7 subfamily B member 1	The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum membrane protein catalyzes the first reaction in the cholesterol catabolic pathway of extrahepatic tissues, which converts cholesterol to bile acids. This enzyme likely plays a minor role in total bile acid synthesis, but may also be involved in the development of atherosclerosis, neurosteroid metabolism and sex hormone synthesis	- Iron ion binding - Oxidation-reduction process - Oxidoreductase activity - Integral component of membrane - Membrane - Metal ion binding - Heme binding - Monooxygenase activity - Endoplastic reticulum membrane - Endoplasmic reticulum - Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen - Positive regulation of epithelial cell proliferation - Bile acid biosynthetic process - Prostate gland epithelium morphogenesis - Negative regulation of intracellular estrogen receptor signaling pathway - Oxysterol 7-alpha-hydroxylase activity
ENSSTOG00000011031	CSAD	cysteine sulfinic acid decarboxylase	This gene encodes a member of the group 2 decarboxylase family. A similar protein in rodents plays a role in multiple biological processes as the rate-limiting enzyme	- Carboxy-lyase activity - Pyridoxal phosphate binding - Lyase activity - Carboxylic acid metabolic process - Catalytic activity - Taurine biosynthetic process

			in taurine biosynthesis, catalyzing the decarboxylation of cysteinesulfinate to hypotaurine	
ENSSTOG00000011808	Missing	Missing		- Integral component of membrane - Membrane - Glucuronosyltransferase activity - Transferase activity, transferring hexosyl groups - Metabolic process - Transferase activity, transferring glycosyl groups - Transferase activity
ENSSTOG00000011815	Missing	Missing		- Integral component of membrane - Membrane - Plasma membrane - Homophilic cell adhesion via plasma membrane adhesion molecules - Cell adhesion - Calcium ion binding - Extracellular exosome - Cytoplasm - Corticospinal tract morphogenesis
ENSSTOG00000012666	Missing	Missing		- Oxidation-reduction process - Oxidoreductase activity - Integral component of membrane - Membrane - Oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water - Fatty acid biosynthetic process

				- Lipid metabolic process
ENSSTOG00000012740	Missing	Missing		- Oxidation-reduction process - Oxidoreductase activity - Integral component of membrane - Membrane - Oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water - Fatty acid biosynthetic process - Lipid metabolic process
ENSSTOG00000014440	SPINT2	serine peptidase inhibitor, Kunitz type, 2	The protein inhibits HGF activator which prevents the formation of active hepatocyte growth factor. This gene is a putative tumor suppressor, and mutations in this gene result in congenital sodium diarrhea	- Integral component of membrane - Membrane - Serine-type endopeptidase inhibitor activity - Negative regulation of endopeptidase activity - Cytoplasm - Negative regulation of cell-cell adhesion - Negative regulation of cell motility - Establishment or maintenance of cell polarity - Neural tube closure - Basement membrane organization - Epithelial cell morphogenesis involved in placental branching
ENSSTOG00000014490	AS3MT	arsenite methyltransferase	AS3MT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (AdoMet) to trivalent arsenical and may play a role in arsenic metabolism	- Arsenite methyltransferase activity - Methylation - Cytosol - Mitochondrion
ENSSTOG00000019453	Missing	Missing		- Integral component of membrane - Membrane - Glucuronosyltransferase activity

				▪ Transferase activity, transferring hexosyl groups ▪ Metabolic process ▪ Transferase activity, transferring glycosyl groups ▪ Transferase activity
ENSSTOG00000021006	FGF21	fibroblast growth factor 21	Theis gene encodes a member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities and are involved in a variety of biological processes. This protein is a secreted endocrine factor that functions as a major metabolic regulator. The encoded protein stimulates the uptake of glucose in adipose tissue	▪ Fibroblast growth factor receptor signaling pathway ▪ Growth factor activity ▪ Fibroblast growth factor receptor binding ▪ Receptor binding ▪ Extracellular region ▪ Protein binding ▪ Positive regulation of ERK1 and ERK2 cascade ▪ Positive regulation of glucose import ▪ Positive regulation of cell proliferation ▪ Positive regulation of MAPKKK cascade by fibroblast growth factor receptor signaling pathway
ENSSTOG00000021833	NRG1	neuregulin 1	The protein encoded by this gene is a membrane glycoprotein that mediates cell-cell signaling and plays a critical role in the growth and development of multiple organ systems.	▪ Integral component of membrane ▪ Membrane ▪ Embryo development ▪ Receptor binding ▪ Positive regulation of cell proliferation ▪ Negative regulation of transcription, DNA-templated ▪ Activation of MAPK activity ▪ Integrin binding ▪ Transcription cofactor activity ▪ Activation of protein kinase B activity

				▪ Positive regulation of protein targeting to mitochondrion
				▪ Negative regultaion of secretion
				▪ ErbB-3 class receptor binding
				▪ Regulation of protein heterodimerization activity
				▪ ERBB signaling pathway
				▪ Endocardial cell differentiation
				▪ Ventricular trabecula myocardium morphogenesis
				▪ Cardiac muscle cell myoblast differentiation
				▪ Cytoplasm
				▪ Nucleus
				▪ Extracellular region
				▪ External side of plasma membrane
				▪ Plasma membrane
				▪ Integral component of plasma membrane
				▪ Protein binding
				▪ Postitive regulation of transcription from RNA polymerase II promoter
				▪ Synapse assembly
				▪ Peripheral nervous system development
				▪ Cell migration
				▪ MAPK cascade
				▪ Postitive regulation of gene expression
				▪ Nervous system development
				▪ Axon
				▪ Brain development
				▪ Positive regulation of Ras protein signal transduction
				▪ Neurogenesis
				▪ Cell morphogenesis
				▪ Startle response
				▪ Cardiac conduction system development

				▪ ErbB-2 class receptor binding
				▪ Heart development
				▪ Muscle organ development
				▪ Locomotory behavior
				▪ Axon ensheathment
				▪ Glial cell differentiation
				▪ Positive regulation of phosphatidylinositol 3-kinase signaling
				▪ Glial cell fate commitment
				▪ Chemorepulsion involved in interneuron migration from the subpallium to the cortex
				▪ Dendrite
				▪ Axolemma
				▪ Neuromuscular junction
				▪ Postive regulation of myelination
				▪ Negative regulation of protein catabolic process
				▪ Cell body
				▪ Synapse
				▪ Neurotransmitter receptor metabolic process
				▪ Chemorepellent activity
				▪ Regulation of cell differentiation
				▪ Positive regulation of axon extension
				▪ Positive regulation of protein kinase activity
				▪ Neuron fate commitment
				▪ Oligodendrocyte differentiation
				▪ Cardio muscle tissue development
				▪ Positive regulation of peptidyl-tyrosine phosphorylation
				▪ Positive regulation of protein kinase B signaling
				▪ Positive regulation of dendritic spine development

				- Positive regulation of calcineurin-NFAT signling cascade - Positive regulation of protein localization to cell surface - Negative regulation of neuron migration - Positive regulation of cardiac muscle cell differentiation
ENSSTOG00000024724	SLC16A11	solute carrier family 16 member 11	Probably involved in hepatic lipid metabolism: overexpression results in an increase of triacylglycerol(TAG) levels, small increases in intracellular diacylglycerols and decreases in lysophosphatidylcholine, cholesterol ester and sphingomyelin lipids	- Integral component of membrane - Membrane - Transmembrane transport - Endoplasmic reticulum membrane - Lipid metabolic process
ENSSTOG00000024978	Missing	Missing		- Transferase activity, transferring hexosyl groups - Integral component of membrane - Membrane - Glucuronosyltransferase activity - Metabolic process - Transferase activity, transferring glycosyl groups - Transferase activity
ENSSTOG00000025261	ANKRD23	ankyrin repeat domain 23	The protein is localized to the nucleus, functioning as a transcriptional regulator. Expression of this protein is	- Cytoplasm - Nucleoplasm - Actin cytoskeleton - Titin binding

			induced during recovery following starvation	
ENSSTOG00000026220	Missing	Missing		▪ Intracellular ▪ Positive regulation of GTPase activity ▪ Guanyl-nucleotide exchange factor activity ▪ Small GTPase mediated signal transduction
ENSSTOG00000026222	Missing	Missing		▪ Integral component of membrane ▪ Membrane ▪ Glucuronosyltransferase activity ▪ Transferase activity, transferring hexosyl groups ▪ Metabolic process ▪ Transferase activity, transferring glycosyl groups ▪ Transferase activity
ENSSTOG00000028144	Missing	Missing		▪ Transferase activity, transferring hexosyl groups ▪ Metabolic process
ENSSTOG00000028862	EEF1A2	eukaryotic translation elongation factor 1 alpha 2	This gene encodes an isoform of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome	▪ GTPase activity ▪ Translational elongation ▪ Translation elongation factor activity ▪ GTP binding ▪ Nucleotide binding ▪ Cytoplasm ▪ Protein binding ▪ Protein kinase binding ▪ Positive regulation of lipid kinase activity ▪ Eukrayotic translation elongation factor 1 complex ▪ Positive regulation of apoptotic process ▪ Myelin sheath
ENSSTOG00000028954	Missing	Missing		▪ Integral component of membrane ▪ Membrane

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				- Glucuronosyltransferase activity - Transferase activity, transferring hexosyl groups - Metabolic process - Transferase activity, transferring glycosyl groups - Transferase activity

Table S4.3 - Over expressed genes in both NY and WV (NYvsMT and COvsWV).

Gene	Name	Description	Gene Cards	GO Terms
ENSSTOG00000001477	LPL	lipoprotein lipase	Expressed in heart, muscle and adipose tissue. Primary function is the hydrolysis of triglycerides of circulating chylomicrons and very low density lipoproteins	▪ Membrane ▪ Plasma membrane ▪ Chylomicron ▪ Very-low-density lipoprotein particle ▪ Anchored component of membrane ▪ Response to glucose ▪ Lipoprotein lipase activity ▪ Heparin binding ▪ Carboxylic ester hydrolase activity ▪ Lipid catabolic process ▪ Lipid metabolic process ▪ Hydrolase activity ▪ Extracellular region ▪ Fatty acid biosynthetic process ▪ Extracellular space ▪ Protein binding ▪ Extracellular exosome ▪ Receptor binding ▪ Positive regulation of sequestering of triglyceride ▪ Triglyceride catabolic process ▪ Cholesterol homeostasis ▪ Positive regulation of chemokine secretion ▪ Apolipoprotein binding ▪ Triglyceride homeostasis ▪ Very-low-density lipoprotein particle remodeling ▪ Positive regulation of cholesterol storage ▪ Triglyceride lipase activity ▪ Cell surface

				▪ Positive regulation of macrophage derived foam cell differentiation
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development.

Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation. | ▪ Membrane
▪ Homophilic cell adhesion via plasma membrane adhesion molecules
▪ Peptidyl-tyrosine phosphorylation
▪ Protein tyrosine kinase activity
▪ Calcium ion binding
▪ Protein phosphorylation
▪ ATP binding
▪ Protein kinase activity
▪ Cytoplasm
▪ Plasma membrane
▪ Protein binding
▪ Integral component of plasma membrane
▪ Receptor complex
▪ Endosome membrane
▪ Intracellular membrane-bounded organelle
▪ Positive regulation of cell migration
▪ Regulation of cell adhesion
▪ Positive regulation of cell adhesion mediated by integrin
▪ Cellular response to reinoic acid
▪ Neuron cell-cell adhesion
▪ Positive regulation of neuron projection development
▪ Membrane protein proteolysis
▪ MAPK cascade
▪ Positive regulation of transcription, DNA templated
▪ Positive regulation of gene expression
▪ Transmembrane receptor protein tyrosine kinase signaling pathway
▪ Nervous system development |

				▪ Positive regulation of cell size ▪ Neuron differentiation ▪ Regulation of axonogenesis ▪ Ureteric bud development ▪ Positive regulation of serine phosphorylation of STAT3 protein ▪ Neural crest cell migration ▪ Enteric nervous system development ▪ Response to pain ▪ Neuron maturation ▪ Embryonic epithelial tube formation ▪ Ureter maturation ▪ Positive regulation of metanephric glomerulus development ▪ Peyer's patch morphogenesis
ENSSTOG00000002475	Missing	Missing		▪ Membrane ▪ Protein binding ▪ Identical protein binding ▪ Synapse ▪ Neuron projection ▪ NMDA glutamate receptor activity ▪ NMDA selective glutamate receptor complex ▪ Neuronal cell body ▪ Glycine binding ▪ Response to ethanol ▪ Postsynaptic membrane ▪ Calcium channel activity ▪ Calcium ion transport ▪ Dendrite development ▪ Prepulse inhibition
ENSSTOG00000003062	TAT	tyrosine aminotransferase	Encodes a mitochondrial protein which is present in the liver and catalyzes the	▪ Aromatic amino acid family catabolic process ▪ Transaminase activity

			conversion of L-tyrosine into p-hydroxyphenylpyruvate	- L-tyrosine:2-oxoglutarate aminotransferase activity - Aromatic amino acid family metabolic process - Cellular amino acid metabolic process - Biosynthetic process - Pyridoxal phosphate binding - Catalytic activity - Tyrosine catabolic process 2-oxoglutarate metabolic process - Gluatamate metabolic process - Mitochondrion
ENSSTOG00000004136	IL1RL2	interleukin 1 receptor like 2	Receptor for interleukin-36. The IL-36 signaling system is thought to be present in epithelial barriers and to take part in local inflammatory responses.	- Integral component of membrane - Membrane - Interleukin-1-mediated signaling pathway - Interleukin-1, Type I, activating receptor activity - Interleukin-1 receptor activity - Cytokine-mediated signaling pathway - Signal transduction - Positive regulation of interleukin-6 production - Positive regulation of T cell differentiation - Regulation of inflammatory response
ENSSTOG00000004982	TRIM63	tripartite motif containing 63	This protein plays an important role in the atrophy of skeletal and cardiac muscle and is required for the degradation of myosin heavy chain proteins, myosin light chain, myosin binding protein, and for	- Intracellular - Metal ion binding - Zinc ion binding - Cytoplasm - Protein binding - Titin binding

			muscle-type creatine kinases.	
ENSSTOG00000005266	PNPLA2	patatin like phospholipase domain containing 2	This gene encodes an enzyme which catalyzes the first step in the hydrolysis of triglycerides in adipose tissue.	▪ Lipid metabolic process ▪ Metabolic process ▪ Lipid particle ▪ Positive regulation of triglyceride catabolic process ▪ Negative regulation of sequestering triglyceride ▪ Protein binding ▪ Cytosol ▪ Lipid storage ▪ Triglyceride catabolic process ▪ Triglyceride lipase activity ▪ Lipid particle organization
ENSSTOG00000008679	CRISPLD1	cysteine rich secretory protein LCCL domain containing 1		▪ Extracellular region ▪ Extracellular exosome ▪ Face morphogenesis
ENSSTOG00000009371	ACE	angiotensin I converting enzyme	Converts angiotensin I to angiotensin II by release of the terminal His-Leu, this results in an increase of the vasoconstrictor activity of angiotensin. Also able to inactivate bradykinin, a potent vasodilator. Has also a glycosidase activity which releases GPI-anchored proteins from the membrane by cleaving the mannose linkage in the GPI moiety	▪ Integral component of membrane ▪ Membrane ▪ Metal ion binding ▪ Peptidyl-dipeptidase activity ▪ Carboxypeptidase activity ▪ Peptidase activity ▪ Hydrolase activity ▪ Metallopeptidase activity ▪ Proteolysis ▪ Plasma membrane ▪ Extracellular space ▪ Zinc ion binding ▪ External side of plasma membrane

				- Extracellular exosome - Endosome - Lysosome - Peptide catabolic process - Drug binding - Endopeptidase activity - Tripeptidyl-peptidase activity - Mitogen-activated protein kinase binding - Kidney development - Arachidnoic acid secretion - Chloride ion binding - Exopeptidease activity - Beta-amyloid metabolic process - Regulation of systemic arterial blood pressure by renin-angiotensin - Mitogen-activated protein kinase kinase binding - Bradykinin receptor binding - Hormone catabolic process - Regulation of angiotensis metabolic process - Metallodipeptidase activity - Cytoplasm - Spermatogenesis - Protein binding - Regulation of blood pressure - Negative regulation of gene expression - Posttranscriptional regulation of gene expression - Negative regulation of protein binding - Positive regulation of protein binding - Positive regulation of protein tyrosine kinase activity - Heart contraction

				- Positive regulation of systemic arterial blood pressure - Positive regulation of peptidyl-tyrosine autophophorylation - Regulation of hematopoietic stem cell proliferation - Neutrophil mediated immunity - Cell proliferation in bone marrow - Negative regulation of gap junction assembly - Positive regulation of peptidyl-cysteine S-nitrosylation
ENSSTOG00000010457	SUSD4	sushi domain containing 4	Acts as complement inhibitor by disrupting the formation of the classical C3 convertase. Isoform 3 inhibits the classical complement pathway, while membrane-bound isoform 1 inhibits deposition of C3b via both the classical and alternative complement pathways.	- Integral component of membrane - Membrane - Regulation of complement activation
ENSSTOG00000012413	IMPA2	inositol monophosphatase 2	This locus encodes an inositol monophosphatase. The encoded protein catalyzes the dephosphoylration of inositol monophosphate and plays an important role in phosphatidylinositol signaling. This locus may be associated with	- Phosphatidylinositol phosphorylation - Cytoplasm - Protein binding - Protein homodimereization activity - Inositol monophosphate 1-phosphatase activity - Inositol phosphate dephosphorylation

			susceptibility to bipolar disorder.	
ENSSTOG00000014794	CTCFL	CCCTC-binding factor like	Testis-specific DNA binding protein responsible for insulator function, nuclear architecture and transcriptional control, which probably acts by recruiting epigenetic chromatin modifiers. Plays a key role in gene imprinting in male germline, by participating in the establishment of differential methylation at the IGF2/H19 imprinted control region (ICR)	- Metal ion binding - Nucleic acid binding - Cytoplasm - Nucleus - DNA binding - RNA polymerase II core promoter proximal region sequence-specific DNA binding - Protein binding - Positive regulation of transcription from RNA polymerase II promoter - Positive regulation of transcription, DNA templated - Positive regulation of gene expression - Transcription regulatory region DNA binding - Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding - Regulation of histone H3-K4 methylation - Sequence-specific DNA binding - Histone binding - Regulation of gene expression by genetic imprinting - Histone methylation - DNA methylation involved in gamete generation
ENSSTOG00000020323	MT1X	metallothionein 1X	Metallothioneins have a high content of cysteine residues that bind various heavy metals; these proteins are transcriptionally regulated	- Metal ion binding - Cytoplasm - Nucleus - Protein binding - Cellular response to erythropoietin - Cellular response to zinc ion

			by both heavy metals and glucocorticoids.	▪ Cellular response to cadmium ion ▪ Cellular zinc ion homeostasis ▪ Nitric oxide mediated signal transduction ▪ Detoxification of copper ion
ENSSTOG00000020350	EIF4EBP3	eukaryotic translation initiation factor 4E binding protein 3	This gene encodes a member of the EIF4EBP family, which consists of proteins that bind to eukaryotic translation initiation factor 4E and regulate its assembly into EIF4F, the multi-subunit translation initiation factor that recognizes the mRNA cap structure.	▪ Negative regulation of translational initiation ▪ Eukaryotic initiation factor 4E binding ▪ Membrane ▪ Protein binding
ENSSTOG00000021209	FSD2	fibronectin type III and SPRY domain containing 2		▪ Protein binding
ENSSTOG00000021636	PPP1R3G	protein phosphatase 1 regulatory subunit 3G	Glycogen-targeting subunit for protein phosphatase 1 (PP1). Involved in the regulation of hepatic glycogenesis in a manner coupled to the fasting-feeding cycle and distinct from other glycogen-targeting subunits	▪ Cytoplasm ▪ Glucose homeostasis ▪ Protein phosphatase binding ▪ Positive regulation of glycogen biosynthetic process ▪ Positive regulation of glycogen (starch) synthase activity ▪ Glycogen binding
ENSSTOG00000022426	TSPAN32	tetraspanin 32	This gene, which is a member of the tetraspanin superfamily, is one of several tumor-suppressing subtransferable fragments located in the imprinted gene	▪ Integral component of membrane ▪ Membrane ▪ Intracellular ▪ Cell surface ▪ Integrin-mediated signaling pathway ▪ Cytoskeleton organization

			domain of chromosome 11p15.5, an important tumor-suppressor gene region.	- Negative regulation of cell proliferation - Regulation of defense response to virus - Defense response to protozoan - Blood coagulation - Platelet aggregation - Hemostasis - Negative regulation of myeloid dendritic cell activation - Integrin alphaIIb-beta3 complex
ENSSTOG00000022668	EVPL	envoplakin	Component of the cornified envelope of keratinocytes. May link the cornified envelope to desmosomes and intermediate filaments. Gene Wiki entry for EVPL Gene	- Intermediate filament binding - Epidermis development - Cornified envelope - Cytoskeleton - Structural molecule activity - Cytoplasm - Extracellular exosome - Protein binding - Peptide cross-linking - Keratinocyte differentiation - Intermediate filament cytoskeleton
ENSSTOG00000024849	Missing	Missing		- Intracellular - Metal ion binding - Cell
ENSSTOG00000026217	SACS	sacsin molecular chaperone	Co-chaperone which acts as a regulator of the Hsp70 chaperone machinery and may be involved in the processing of other ataxia-linked proteins.	- Cytoplasm - Nucleus - Mitochondrion - Proteasome binding - Chaperone binding - Hsp70 protein binding - Negative regulation of inclusion body assembly
ENSSTOG00000026420	FBP1	fructose-bisphosphatase 1	Catalyzes the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate in the	- Fructose 1,6-bisphosphate 1-phosphatase activity - Phosphoric ester hydrolase activity

			presence of divalent cations, acting as a rate-limiting enzyme in gluconeogenesis. Plays a role in regulating glucose sensing and insulin secretion of pancreatic beta-cells. Appears to modulate glycerol gluconeogenesis in liver. Important regulator of appetite and adiposity; increased expression of the protein in liver after nutrient excess increases circulating satiety hormones and reduces appetite-stimulating neuropeptides and thus seems to provide a feedback mechanism to limit weight gain.	- Hydrolase activity - Carbohydrate metabolic process - Cytoplasm - Metal ion binding - Protein binding - Identical protein binding - Extracellular exosome - Dephosphoylation - Negative regulation of Ras protein signal transduction - Gluconeogenesis - Negative regulation of cell growth - Cellular response to drug - Protein homotetramerization - Negative regulation of glycolytic process - Fructose 6-phosphate metabolic process - AMP binding - Regulation of gluconeogenesis - Cellular response to magnesium ion
ENSSTOG00000027573	COMT	catechol-O-methyltransferase	Catalyzes the O-methylation, and thereby the inactivation, of catecholamine neurotransmitters and catechol hormones. Also shortens the biological half-lives of certain neuroactive drugs, like L-DOPA, alpha-methyl DOPA and isoproterenol.	- Integral component of membrane - Membrane - Neurotransmitter catabolic process - Catechol O-methyltransferase activity - Catecholamine metabolic process - O-methyltransferase activity - Methylation - Magnesium ion binding - Protein binding - Extracellular exosome - Mitochondrion - Dopamine metabolic process - Catechol-containing compound metabolic process

				- Cellular response to phosphate starvation - Dopamine catabolic process
ENSSTOG00000028432	Missing	Missing		- Metabolic process - Catalytic activity - Mitochondrion

Table S4.4 - Over expressed genes in both NY winter and MT (NYwvsNYs and NYvsMT).

Gene	Name	Description	Gene Cards	GO Terms
ENSSTOG00000007640	ID1	inhibitor of DNA binding 1, HLH protein		- Negative regulation of transcription, DNA-templated - Cytoplasm - Protein dimerization activity - Nucleus - Protein binding - Nucleoplasm - Golgi apparatus - Negative regulation of sequence-specific DNA binding transcription factor activity - Transcription factor activity, sequence-specific DNA binding - Transcription, DNA templated - Negative regulation of apoptotic process - Negative regulation of gene expression - Negative regulation of transcription from RNA polymerase II promoter - Negative regulation of DNA binding - Heart development - Circadian rhythm - Protein self-association - Endothelial cell morphogenesis - Regulation of angiogenesis - Collagen metabolic process - BMP signaling pathway - Negative regulation of osteoblast differentiation - Regulation of MAPK cascade - Lung morphogenesis - Reponse to antibiotic - Protein destabilization

				▪ Lung vasculature development ▪ Negative regulation of protein homodimerization activity
ENSSTOG00000011815	Missing	Missing		▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Cell adhesion ▪ Calcium ion binding ▪ Extracellular exosome ▪ Cytoplasm ▪ Corticospinal tract morphogenesis
ENSSTOG00000019401	ID2	inhibitor of DNA binding 2, HLH protein		▪ Negative regulation of transcription, DNA-templated ▪ Cytoplasm ▪ Protein dimerization activity ▪ Nucleus ▪ Cytosol ▪ Protein binding ▪ Nucleoplasm ▪ Ion channel binding ▪ Centrosome ▪ Negative regulation of sequence-specific DNA binding transcription factor activity ▪ Regulation of G1/S transition of mitotic cell cycle ▪ Protein complex ▪ Positive regulation of transcription, DNA templated ▪ Positive regulation of gene expression ▪ Negative regulation of gene expression ▪ Negative regulation of transcription from RNA polymerase II promoter

				- Negative regulation of DNA binding
				- Heart development
				- Neuron fate commitment
				- Locomotor rhythm
				- Adipose tissue development
				- Positive regulation of fat cell differentiation
				- Oligodendrocyte development
				- Olfactory bulb development
				- Circadian rhythm
				- Cell maturation
				- Metanephros development
				- Circadian regulation of gene expression
				- Adult locomotory behavior
				- Negative regulation of neuron differentiation
				- Cell development
				- Positive regulation of blood pressure
				- Negative regulation of neural precursor cell proliferation
				- Negative regulation of osteoblast differentiation
				- Regulation of lipid metabolic process
				- Natural killer cell differentiation
				- Positive regulation of astrocyte differentiation
				- Regulation of circadian rhythm
				- Positive regulation of erythrocyte differentiation
				- Mammary gland alveolus development
				- Entrainment of circadian clock by photoperiod
				- Mammary gland epithelial cell proliferation
				- Positive regulation of cell cycle arrest
				- Embryonic digestive tract morphogenesis
				- Leukocyte differentiation

				▪ Negative regulation of B cell differentiation ▪ Positive regulation of smooth muscle cell proliferation ▪ Membranous septum morphogenesis ▪ Enucleate erythrocyte differentiation ▪ Positive regulation of macrophage differentiation ▪ Peyer's patch development ▪ Cellular response to lithium ion ▪ Cell morphogenesis involved in neuron differentiation ▪ Cellular senescence ▪ Endodermal digestive tract morphogenesis ▪ Negative regulation of oligodendrocyte differentiation ▪ Thigmotaxis ▪ Bundle of His development ▪ Entrainment of circadian clock ▪ Epithelial cell differentiation involved in mammary gland alveolus development
ENSSTOG00000020259	Missing	Missing		▪ Hydrolase activity, hydrolyzing O-glycosyl compounds ▪ Carbohydrate metabolic process
ENSSTOG00000024534	Missing	Missing		▪ Metal ion binding
ENSSTOG00000024583	CISH	cytokine inducible SH2 containing protein	The expression of this gene can be induced by IL2, IL3, GM-CSF and EPO in hematopoietic cells . Proteasome-mediated degradation of this protein has been shown to be involved in the inactivation	▪ Intracellular ▪ Protein ubiquitination ▪ Intracellular signal transduction ▪ Protein binding ▪ Plasma membrane ▪ Protein kinase C-activating G-protein coupled receptor signaling pathway

			of the erythropoietin receptors.	

Table S4.5 - Over expressed genes in NY winter (NY winter and NY summer comparison)

Gene	Name	Description	Gene cards	GO Terms
ENSSTOG00000000294	ABCA4	ATP binding cassette subfamily A member 4		▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ Transmembrane transport ▪ ATPase activity, coupled to transmembrane movement of substances ▪ ATPase activity ▪ Integral component of plasma membrane ▪ Transporter activity ▪ Visual perception ▪ ATP binding ▪ Nucleotide binding ▪ Photoreceptor outer segment ▪ Phospholipid-translocating ATPase sensitivity ▪ Phospholipid transporter activity ▪ Photoreceptor cell maintenance ▪ Phospholipid transfer to membrane
ENSSTOG00000002629	KDELR3	KDEL endoplasmic reticulum protein retention receptor 3		▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ ER retention sequence binding ▪ Protein retention in ER lumen ▪ Protein transport ▪ Endoplasmic reticulum membrane ▪ Endoplasmic reticulum
ENSSTOG00000003005	FOS	Fos proto-oncogene, AP-1 transcription factor subunit		▪ DNA Binding ▪ Transcription factor complex ▪ Sequence-specific DNA binding

				- Transcription from RNA polymerase II promoter - Transcription factor activity, sequence-specific DNA binding - Regulation of transcription from RNA polymerase II promoter Regulation of transcription, DNA-templated - Nucleus - Protein binding - Positive regulation of transcription from RNA polymerase II promoter - Positive regulation of transcription, DNA templated - Nucleoplasm - Transcription factor binding - Transcription regulatory region DNA binding - R-SMAD binding - Transforming growth factor beta receptor signaling pathway - SMAD protein signal transduction - Cellular response to reactive oxygen species - Chromatin binding - RNA polymerase II core promoter proximal region sequence-specific DNA binding - Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding - Skeletal muscle cell differentiation - Nervous system development - Response to drug - RNA polymerase II core promoter sequence-specific DNA binding - Cellular response to calcium ion - Response to muscle stretch

				▪ Positive regulation of osteoclast differentiation ▪ Cellular response to extracellular stimulus
ENSSTOG00000003590	ROPN1L	rhophilin associated tail protein 1 like		▪ Cytoplasm ▪ Protein binding ▪ Motile cilium
ENSSTOG00000004163	MAGED1	MAGE family member D1		▪ Circadian regulation of gene expression ▪ Regulation of apoptotic process ▪ Protein binding ▪ Protein complex ▪ Negative regulation of epithelial cell proliferation ▪ Nucleus ▪ Negative regulation of transcription, DNA templated ▪ Chromatin ▪ Positive regulation of transcription, DNA-templated ▪ Regulation of transcription from RNA polymerase II promoter ▪ Transcription coactivator activity ▪ Positive regulation of branching involved in ureteric bud morphogenesis ▪ Positive regulation of apoptotic signaling pathway ▪ Regulation of circadian rhythm ▪ Postive regulation of MAP kinase activity
ENSSTOG00000006538	ATOH8	atonal bHLH transcription factor 8		▪ Cell differentiation ▪ Transcription factor activity, sequence-specific DNA binding ▪ Protein dimerization activity Regulation of transcription, DNA templated ▪ Nucleus

				▪ Positive regulation of transcription, DNA templated ▪ SMAD protein signal transduction ▪ E-box binding ▪ Negative regulation of endothelial cell proliferation ▪ Postive regulation of endothelial cell migration ▪ Tube formation ▪ Postive regulation of endothelial cell differentiation ▪ Cytoplasm ▪ Transcription factor binding ▪ Negative regulation of transcription, DNA templated ▪ Activating transcription factor binding ▪ Formation of primary germ layer ▪ Myoblast proliferation
ENSSTOG00000007587	NFKBIA	NFKB inhibitor alpha		▪ Cytoplasm ▪ Plasma membrane ▪ Nucleus ▪ Protein binding ▪ Positive regulation of transcription from RNA polymerase II promoter Identical protein binding ▪ Ubiquitin protein ligase binding ▪ Negative regulation of NF-kappB transcription factor activity ▪ Nuclear locatlization sequence binding ▪ Enzyme binding ▪ Cytoplasmic sequestering of transcription factor

				▪ NF-kappaB binding Positive regulation of cellular protein metabolic process ▪ Cytoplasmic sequestering of NF-kappaB ▪ Negative regulation of macrophage derived foam cell differentiation ▪ Positive regulation of cholesterol efflux ▪ Negative regulation of lipid storage ▪ Regulation of gene expression ▪ Cytosol ▪ Postitive regulation of transcription, DNA templated ▪ Regulation of cell proliferation ▪ Response to Lipopolysaccharide ▪ Response to muramyl dipeptide ▪ Response to exogenous dsRNA ▪ Lipopolysaccharide-mediated signaling pathway ▪ Toll-like receptor 4 signaling pathway ▪ Negative regulation of myeloid cell differentiation ▪ Response to muscle stretch ▪ Negative regulation of Notch signaling pathway ▪ Protein import into nucleus, translocation ▪ Nucleotide-binding oligomerization domain containing 1 signaling pathway ▪ Nucleotide-binding oligomerization domain containing 2 signaling pathway
ENSSTOG00000007598	SLC6A1	solute carrier family 6 member 1		▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ Transmembrane transport ▪ Gamma-aminobutyric acid transport

				▪ Neurotransmitter transport ▪ Gamma-aminobutyric acid:sodium symporter activity ▪ Neurotransmitter:sodium symporter activity ▪ Symporter activity ▪ Integral component of plasma membrane ▪ Mitophagy in response to mitochondrial depolarization ▪ Plasma membrane ▪ Axon
ENSSTOG00000007640	ID1	inhibitor of DNA binding 1, HLH protein		▪ Negative regulation of transcription, DNA-templated ▪ Cytoplasm ▪ Protein dimerization activity ▪ Nucleus ▪ Protein binding ▪ Nucleoplasm ▪ Golgi apparatus ▪ Negative regulation of sequence-specific DNA binding transcription factor activity ▪ Transcription factor activity, sequence-specific DNA binding ▪ Transcription, DNA templated ▪ Negative regulation of apoptotic process ▪ Negative regulation of gene expression ▪ Negative regulation of transcription from RNA polymerase II promoter ▪ Negative regulation of DNA binding ▪ Heart development ▪ Circadian rhythm ▪ Protein self-association ▪ Endothelial cell morphogenesis ▪ Regulation of angiogenesis ▪ Collagen metabolic process

			• BMP signaling pathway • Negative regulation of osteoblast differentiation • Regulation of MAPK cascade • Lung morphogenesis • Reponse to antibiotic • Protein destabilization • Lung vasculature development
ENSSTOG00000011295	SAG	S-antigen; retina and pineal gland (arrestin)	• Signal transduction • Photoreceptor outer segment, opsin binding, phosphoprotein binding • Photoreceptor inner segment
ENSSTOG00000011815	Missing	Missing	• Calcium ion binding • Integral component of membrane • Membrane • Plasma membrane • Homophilic cell adhesion via plasma membrane adhesion molecules • Cell adhesion • Extracellular exosome • Cytoplasm • Corticospinal tract morphogenesis
ENSSTOG00000012423	TG	thyroglobulin	• Thyroid gland development • Extracellular space • Thyroid hormone metabolic process • Regulation of myelination • Iodide transport
ENSSTOG00000019401	ID2	inhibitor of DNA binding 2, HLH protein	• Negative regulation of transcription, DNA-templated • Cytoplasm • Protein dimerization activity • Nucleus • Cytosol • Protein binding

				▪ Nucleoplasm
				▪ Ion channel binding
				▪ Centrosome
				▪ Negative regulation of sequence-specific DNA binding transcription factor activity
				▪ Regulation of G1/S transition of mitotic cell cycle
				▪ Protein complex
				▪ Positive regulation of transcription, DNA templated
				▪ Positive regulation of gene expression
				▪ Negative regulation of gene expression
				▪ Negative regulation of transcription from RNA polymerase II promoter
				▪ Negative regulation of DNA binding
				▪ Heart development
				▪ Neuron fate commitment
				▪ Locomotor rhythm
				▪ Adipose tissue development
				▪ Positive regulation of fat cell differentiation
				▪ Oligodendrocyte development
				▪ Olfactory bulb development
				▪ Circadian rhythm
				▪ Cell maturation
				▪ Metanephros development
				▪ Circadian regulation of gene expression
				▪ Adult locomotory behavior
				▪ Negative regulation of neuron differentiation
				▪ Cell development
				▪ Positive regulation of blood pressure
				▪ Negative regulation of neural precursor cell proliferation
				▪ Negative regulation of osteoblast differentiation

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				- Regulation of lipid metabolic process - Natural killer cell differentiation - Positive regulation of astrocyte differentiation - Regulation of circadian rhythm - Positive regulation of erythrocyte differentiation - Mammary gland alveolus development - Entrainment of circadian clock by photoperiod - Mammary gland epithelial cell proliferation - Positive regulation of cell cycle arrest - Embryonic digestive tract morphogenesis - Leukocyte differentiation - Negative regulation of B cell differentiation - Positive regulation of smooth muscle cell proliferation - Membranous septum morphogenesis - Enucleate erythrocyte differentiation - Positive regulation of macrophage differentiation - Peyer's patch development - Cellular response to lithium ion - Cell morphogenesis involved in neuron differentiation - Cellular senescence - Endodermal digestive tract morphogenesis - Negative regulation of oligodendrocyte differentiation - Thigmotaxis - Bundle of His development - Entrainment of circadian clock - Epithelial cell differentiation involved in mammary gland alveolus development

ENSSTOG00000020259	Missing	Missing		▪ Hydrolase activity, hydrolyzing O-glycosyl compounds ▪ Carbohydrate metabolic process
ENSSTOG00000021197	Missing	Missing		▪ Calcium ion binding ▪ Cytoplasm ▪ Nucleus ▪ Membrane ▪ Protein binding ▪ Nucleolus Golgi apparatus ▪ Positive regulation of calcium ion transport ▪ Activation of store-operated calcium channel activity ▪ Store-operated calcium entry
ENSSTOG00000021898	CYP26B1	cytochrome P450 family 26 subfamily B member 1		▪ Iron ion binding ▪ Oxidation-reduction process ▪ Oxidoreductase activity ▪ Metal ion binding ▪ Heme binding ▪ Monooxygenase activity ▪ Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen ▪ Cytoplasm ▪ Bone morphogenesis Retinoic acid binding ▪ Retinoic acid 4-hydroxylase activity ▪ Retinoic acid catabolic process ▪ Spermatogenesis ▪ Inflammatory response ▪ Positive regulation of gene expression ▪ Negative regulation of retinoic acid receptor signaling pathway ▪ Establishment of skin barrier

				- Cellular response to retinoic acid - Male meiosis - Retinoic acid receptor signaling pathway - Regulation of T cell differentiation - Cell fate determination - Embryonic limb morphogenesis - Proximal/distal pattern formation - Establishment of T cell polarity - Cornification - Regulation of retinoic acid receptor signaling pathway - Tongue morphogenesis - Positive regulation of tongue muscle cell differentiation
ENSSTOG00000024485	TMEM200C	transmembrane protein 200C		- Integral component of membrane - Membrane
ENSSTOG00000024534	Missing	Missing		Metal ion binding
ENSSTOG00000024583	CISH	cytokine inducible SH2 containing protein	The expression of this gene can be induced by IL2, IL3, GM-CSF and EPO in hematopoietic cells . Proteasome-mediated degradation of this protein has been shown to be involved in the inactivation of the erythropoietin receptors.	- Intracellular - Protein ubiquitination - Intracellular signal transduction - Protein binding - Plasma membrane - Protein kinase C-activating G-protein coupled receptor signaling pathway
ENSSTOG00000027370	GADD45G	growth arrest and DNA damage inducible gamma		- Response to stress - Regulation of cell cycle - Nucleus

				- Cytoplasm Protein binding - Postive regulation of apoptotic process - Positive regulation of JNK cascade - Activation of MAPKKK activity - Positive regulation of p38MAPK cascade

Table S4.6 - Over expressed genes in CO and NY winter (CO vs WV and NYw vs NYs)

Gene	Name	Description	Gene card	GO Terms
ENSSTOG00000011815	Missing	Missing		- Calcium ion binding - Integral component of membrane - Membrane - Plasma membrane - Homophilic cell adhesion via plasma membrane adhesion molecules cell adhesion - Extracellular exosome - Cytoplasm - Corticospinal tract morphogenesis

Table S4.7 - Over expressed genes in NY & NY (NYs vs NYw and NYs vs MT)

Gene	Name	Description	Gene card	GO Terms
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development. Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation.	▪ Membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Peptidyl-tyrosine phosphorylation ▪ Protein tyrosine kinase activity ▪ Calcium ion binding ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Cytoplasm ▪ Plasma membrane ▪ Protein binding ▪ Integral component of plasma membrane ▪ Receptor complex ▪ Endosome membrane ▪ Intracellular membrane-bounded organelle ▪ Positive regulation of cell migration ▪ Regulation of cell adhesion ▪ Positive regulation of cell adhesion mediated by integrin ▪ Cellular response to reinoic acid ▪ Neuron cell-cell adhesion ▪ Positive regulation of neuron projection development ▪ Membrane protein proteolysis ▪ MAPK cascade ▪ Positive regulation of transcription, DNA templated ▪ Positive regulation of gene expression ▪ Transmembrane receptor protein tyrosine kinase signaling pathway

				▪ Nervous system development ▪ Positive regulation of cell size ▪ Neuron differentiation ▪ Regulation of axonogenesis ▪ Ureteric bud development ▪ Positive regulation of serine phophoylation of STAT3 protein ▪ Neural crest cell migration ▪ Enteric nervous system development ▪ Response to pain ▪ Neuron maturation ▪ Embryonic epithelial tube formation ▪ Ureter maturation ▪ Positive regulation of metanephric glomerulus development ▪ Peyer's patch morphogenesis
ENSSTOG00000005711	IRF7	interferon regulatory factor 7		▪ Regulatory region DNA binding ▪ Transcription factor activity, sequence-specific DNA binding ▪ Regulation of transcription, DNA-templated ▪ Cytoplasm ▪ Nucleus ▪ DNA binding ▪ Protein binding ▪ Positive regulation of transcription from RNA polymerase II promoter ▪ Positive regulation of transcription, DNA-templated ▪ Nucleoplasm ▪ Positive regulation of interferon-alpha production ▪ Positive regulation of interferon-beta production

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				▪ Transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding ▪ RNA polymerase II core promoter sequence-specific DNA binding ▪ Regulation of adaptive immune response ▪ Immunoglobulin mediated immune response ▪ Positive regulation of type I interferon-mediated signaling pathway ▪ Positive regulation of type I interferon production ▪ Regulation of MyD88-dependent toll-like receptor signaling pathway ▪ Type I interferon biosynthetic process ▪ Interferon-alpha production ▪ Interferon-beta production ▪ Regulation of MyD88-independent toll-like receptor signaling pathway
ENSSTOG00000007829	TNNI2	troponin I2, fast skeletal type		▪ Troponin complex ▪ Nucleus ▪ Actin binding ▪ Protein binding ▪ Positive regulation of transcription ▪ DNA-templated ▪ Skeletal muscle contraction ▪ Troponin T binding
ENSSTOG00000010414	PALLD	palladin, cytoskeletal associated protein		▪ Integral component of membrane ▪ Membrane ▪ Cell migration ▪ Actin cytoskeleton organization ▪ Actin binding ▪ Epithelial cell morphogenesis ▪ Cytoplasm ▪ Plasma membrane

				- Nucleus - Mitochondrion - Protein binding - Actin cytoskeleton - Actin filament - Focal adhesion - Epithelial cell morphogenesis - Keratinocyte development
ENSSTOG00000020847	Missing	Missing		- Thiol-dependent ubiquitinyl hydrolase activity - Protein deubiquitination - Ubiquitin-dependent protein catabolic process - Protein binding - Response to stilbenoid - ISG15-specific protease activity
ENSSTOG00000025690	SYT8	synaptotagmin 8		- Integral component of membrane - Membrane - Exocytosis - Cytoplasm - Acrosome reaction - Calcium-dependent protein binding - Membrane-bounded vesicle
ENSSTOG00000028109	CPNE6	copine 6		- Protein binding - Extracellular exosome - Positive regulation of dendrite extension - Cytoplasm - Plasma membrane - Membrane - Axon - Dendrite - Cellular response to calcium ion - Phosphatidylserine binding - Clathrin-coated endocytic vesicle

ENSSTOG00000028432	Missing	Missing		▪ Metabolic process ▪ Catalytic activity ▪ Mitochondrion

Table S4.8 - Over expressed genes in NY and NY (NY vs AK and NY vs MT)

Gene	Name	Description	Gene card	GO Terms
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development. Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation.	• Membrane • Homophilic cell adhesion via plasma membrane adhesion molecules • Peptidyl-tyrosine phosphorylation • Protein tyrosine kinase activity • Calcium ion binding • Protein phosphorylation • ATP binding • Protein kinase activity • Cytoplasm • Plasma membrane • Protein binding • Integral component of plasma membrane • Receptor complex • Endosome membrane • Intracellular membrane-bounded organelle • Positive regulation of cell migration • Regulation of cell adhesion • Positive regulation of cell adhesion mediated by integrin • Cellular response to reinoic acid • Neuron cell-cell adhesion • Positive regulation of neuron projection development • Membrane protein proteolysis • MAPK cascade • Positive regulation of transcription, DNA templated • Positive regulation of gene expression • Transmembrane receptor protein tyrosine kinase signaling pathway

				▪ Nervous system development ▪ Positive regulation of cell size ▪ Neuron differentiation ▪ Regulation of axonogenesis ▪ Ureteric bud development ▪ Positive regulation of serine phophoylation of STAT3 protein ▪ Neural crest cell migration ▪ Enteric nervous system development ▪ Response to pain ▪ Neuron maturation ▪ Embryonic epithelial tube formation ▪ Ureter maturation ▪ Positive regulation of metanephric glomerulus development ▪ Peyer's patch morphogenesis
ENSSTOG00000003810	Missing	Missing		▪ Protein serine/threonine kinase activity ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Nucleotide binding ▪ Cytoplasm ▪ Nucleus ▪ Magnesium ion binding ▪ Protein binding ▪ Intracellular signal transduction ▪ Protein kinase binding ▪ Regulation of mitotic cell cycle ▪ Anoikis ▪ Regulation of cell differentiation ▪ Histone deacetylase binding ▪ Cardiac muscle cell differentiation ▪ Regulation of myotube differentiation ▪ Negative regulation of gluconeogenesis

				▪ Entrainment of circadian clock by photoperiod ▪ cAMP response element binding protein binding ▪ Negative regulation of CREB transcription factor activity ▪ Negative regulation of triglyceride biosynthetic process
ENSSTOG00000004982	TRIM63	tripartite motif containing 63	This protein plays an important role in the atrophy of skeletal and cardiac muscle and is required for the degradation of myosin heavy chain proteins, myosin light chain, myosin binding protein, and for muscle-type creatine kinases.	▪ Intracellular ▪ Metal ion binding ▪ Zinc ion binding ▪ Cytoplasm ▪ Protein binding ▪ Titin binding
ENSSTOG00000006748	ESR1	estrogen receptor 1		▪ DNA binding ▪ Metal ion binding ▪ Intracellular estrogen receptor signaling pathway ▪ Estrogen receptor activity ▪ Steroid binding ▪ Intracellular receptor signaling pathway ▪ Lipid binding ▪ Steroid hormone mediated signaling pathway ▪ Steroid hormone receptor activity ▪ Sequence-specific DNA binding ▪ Transcription factor activity, sequence-specific DNA binding ▪ Transcription, DNA-templated ▪ Zinc ion binding

				▪ Regulation of transcription, DNA-templated ▪ Nucleus ▪ Cytoplasm ▪ Plasma membrane ▪ Negative regulation of transcription from RNA polymerase II promoter ▪ RNA polymerase II core promoter proximal region sequence-specific DNA binding ▪ Protein binding ▪ Positive regulation of transcription from RNA polymerase II promoter ▪ Identical protein binding ▪ Transcription from RNA polymerase II promoter ▪ Positive regulation of transcription, DNA-templated ▪ Chromatin binding ▪ Transcription factor binding ▪ Beta-catenin binding ▪ Negative regulation of gene expression ▪ Enzyme binding ▪ Nuclear chromatin ▪ Negative regulation of sequence-specific DNA binding transcription factor activity ▪ Positive regulation of sequence-specific DNA binding transcription factor activity ▪ Core promoter sequence-specific DNA binding ▪ ATPase binding ▪ Transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding ▪ Response to estradiol ▪ Response to estrogen

				▪ Estrogen response element binding
				▪ Negative regulation of I-kappaB kinase/NF-kappaB signaling
				▪ Protein localization to chromatin
				▪ Cellular response to estradiol stimulus
				▪ Negative regulation of production of miRNAs involved in gene silencing by miRNA
				▪ Transcriptionally active chromatin
				▪ RNA polymerase II transcription factor activity, estrogen-activated sequence-specific DNA-binding
				▪ Regulation of apoptotic process
				▪ RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding
				▪ Epithelial cell development
				▪ Male gonad development
				▪ Regulation of inflammatory response
				▪ Uterus development
				▪ Vagina development
				▪ Regulation of toll-like receptor signaling pathway
				▪ Mammary gland alveolus development
				▪ Positive regulation of fibroblast proliferation
				▪ Prostate epithelial cord arborization involved in prostate glandular acinus morphogenesis
				▪ Cellular response to estrogen stimulus
				▪ Antral ovarian follicle growth
				▪ Androgen metabolic process
				▪ Prostate epithelial cord elongation
				▪ Regulation of branching involved in prostate gland morphogenesis

				▪ Mammary gland branching involved in pregnancy ▪ Epithelial cell proliferation involved in mammary gland duct elongation
ENSSTOG00000008679	CRISPL D1	cysteine rich secretory protein LCCL domain containing 1		▪ Extracellular region ▪ Extracellular exosome ▪ Face morphogenesis
ENSSTOG00000011601	Missing	Missing		▪ Ion transport ▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ Transmembrane transport ▪ Regulation of postsynaptic membrane potential ▪ Sodium ion transmembrane transport ▪ Sodium ion transport ▪ Sodium channel activity ▪ Voltage-gated sodium channel activity ▪ Voltage-gated sodium channel complex ▪ Ion transmembrane transport ▪ Regulation of ion transmembrane transport ▪ Voltage-gated ion channel activity ▪ Ion channel activity
ENSSTOG00000012159	MYRIP	myosin VIIA and Rab interacting protein		▪ Protein binding ▪ Actin binding ▪ Actin cytoskeleton ▪ Rab GTPase binding ▪ Photoreceptor outer segment ▪ Exocyst ▪ Synapse ▪ Protein kinase A binding ▪ Myosin binding

				▪ Melanosome
ENSSTOG00000014041	MYO5C	myosin VC		▪ Metal ion binding ▪ Myosin complex ▪ ATP binding ▪ Actin binding ▪ Motor activity ▪ Nucleotide binding ▪ Extracellular exosome
ENSSTOG00000020834	C6orf132	chromosome 6 open reading frame 132		
ENSSTOG00000021507	Missing	Missing		▪ Glutathione transferase activity ▪ Metabolic process
ENSSTOG00000021636	PPP1R3G	protein phosphatase 1 regulatory subunit 3G	Glycogen-targeting subunit for protein phosphatase 1 (PP1). Involved in the regulation of hepatic glycogenesis in a manner coupled to the fasting-feeding cycle and distinct from other glycogen-targeting subunits	▪ Cytoplasm ▪ Glucose homeostasis ▪ Protein phosphatase binding ▪ Positive regulation of glycogen biosynthetic process ▪ Positive regulation of glycogen (starch) synthase activity ▪ Glycogen binding
ENSSTOG00000022426	TSPAN32	tetraspanin 32	This gene, which is a member of the tetraspanin superfamily, is one of several tumor-suppressing subtransferable fragments located in the imprinted gene domain of chromosome 11p15.5, an important tumor-suppressor gene region.	▪ Integral component of membrane ▪ Membrane ▪ Intracellular ▪ Cell surface ▪ Integrin-mediated signaling pathway ▪ Cytoskeleton organization ▪ Negative regulation of cell proliferation ▪ Regulation of defense response to virus ▪ Defense response to protozoan ▪ Blood coagulation

				• Platelet aggregation • Hemostasis • Negative regulation of myeloid dendritic cell activation • Integrin alphaIIb-beta3 complex
ENSSTOG00000022668	EVPL	envoplakin	Component of the cornified envelope of keratinocytes. May link the cornified envelope to desmosomes and intermediate filaments. Gene Wiki entry for EVPL Gene	• Intermediate filament binding • Epidermis development • Cornified envelope • Cytoskeleton • Structural molecule activity • Cytoplasm • Extracellular exosome • Protein binding • Peptide cross-linking • Keratinocyte differentiation • Intermediate filament cytoskeleton
ENSSTOG00000024424	Missing	Missing		• Exocytosis • Vesicle docking • Cytoplasm • Ral GTPase binding • Protein N-terminus binding
ENSSTOG00000024594	A3GALT 2	alpha 1,3-galactosyltransferase 2		• Integral component of membrane • Membrane • Transferase activity, transferring hexosyl groups • Carbohydrate metabolic process • Alpha-1,3-galactosyltransferase activity • Glycosphingolipid biosynthetic process
ENSSTOG00000026217	SACS	sacsin molecular chaperone	Co-chaperone which acts as a regulator of the Hsp70 chaperone machinery and may be involved in the	• Cytoplasm • Nucleus • Mitochondrion • Proteasome binding • Chaperone binding • Hsp70 protein binding

			processing of other ataxia-linked proteins.	• Negative regulation of inclusion body assembly
ENSSTOG00000027573	COMT	catechol-O-methyltransferase	Catalyzes the O-methylation, and thereby the inactivation, of catecholamine neurotransmitters and catechol hormones. Also shortens the biological half-lives of certain neuroactive drugs, like L-DOPA, alpha-methyl DOPA and isoproterenol.	• Integral component of membrane • Membrane • Neurotransmitter catabolic process • Catechol O-methyltransferase activity • Catecholamine metabolic process • O-methyltransferase activity • Methylation • Magensium ion binding • Protein binding • Extracellular exosome • Mitochondrion • Dopamine metabolic process • Catechol-containing compound metabolic process • Cellular response to phosphate starvation • Dopamine catabolic process
ENSSTOG00000027585	LGR6	leucine rich repeat containing G protein-coupled receptor 6		• Integral component of membrane • Membrane • Protein-hormone receptor activity • G-protein coupled receptor signaling pathway • G-protein coupled receptor activity • Protein binding • Positive regulation of cell migration • Vesicle • Positive regulation of Wnt signaling pathway
ENSSTOG00000028432	Missing	Missing		• Metabolic process • Catalytic activity • Mitochondrion

Table S4.9 - Over expressed genes in NY summer (NYs and NYw)

Gene	Name	Description	Gene card	GO Terms
ENSSTOG00000007820	Missing	Missing		▪ Integral component of membrane ▪ Membrane ▪ Carbohydrate binding ▪ Golgi membrane ▪ Transferase activity, transferring glycosyl groups ▪ Protein glycosylation ▪ Golgi apparatus ▪ Transferase activity
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development. Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation.	▪ Membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Peptidyl-tyrosine phosphorylation ▪ Protein tyrosine kinase activity ▪ Calcium ion binding ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Cytoplasm ▪ Plasma membrane ▪ Protein binding ▪ Integral component of plasma membrane ▪ Receptor complex ▪ Endosome membrane ▪ Intracellular membrane-bounded organelle ▪ Positive regulation of cell migration ▪ Regulation of cell adhesion ▪ Positive regulation of cell adhesion mediated by integrin ▪ Cellular response to reinoic acid

				- Neuron cell-cell adhesion - Positive regulation of neuron projection development - Membrane protein proteolysis - MAPK cascade - Positive regulation of transcription, DNA templated - Positive regulation of gene expression - Transmembrane receptor protein tyrosine kinase signaling pathway - Nervous system development - Positive regulation of cell size - Neuron differentiation - Regulation of axonogenesis - Ureteric bud development - Positive regulation of serine phophoylation of STAT3 protein - Neural crest cell migration - Enteric nervous system development - Response to pain - Neuron maturation - Embryonic epithelial tube formation - Ureter maturation - Positive regulation of metanephric glomerulus development - Peyer's patch morphogenesis
ENSSTOG00000025236	CFAP100	cilia and flagella associated protein 100		
ENSSTOG00000019659	FAM111A	family with sequence		- Cytoplasm - Nucleus - DNA replication - Protein binding

		similarity 111 member		▪ Negative regulation of viral genome replication ▪ Chromatin
ENSSTOG00000004459	Missing	Missing		▪ Integral component of membrane
ENSSTOG00000011224	TINAG	tubulointerstitial nephritis antigen		▪ Polysaccharide binding ▪ Scavenger receptor activity ▪ Cysteine-type peptidase activity ▪ Immune response ▪ Receptor-mediated endocytosis ▪ Proteolysis ▪ Cell adhesion ▪ Basement membrane
ENSSTOG00000028109	CPNE6	copine 6		▪ Protein binding ▪ Extracellular exosome ▪ Positive regulation of dendrite extension ▪ Cytoplasm ▪ Plasma membrane ▪ Membrane ▪ Axon ▪ Dendrite ▪ Cellular response to calcium ion ▪ Phosphatidylserine binding ▪ Clathrin-coated endocytic vesicle
ENSSTOG00000025690	SYT8	synaptotagmin 8		
ENSSTOG00000024345	EBI3	Epstein-Barr virus induced 3		▪ Membrane ▪ Cytokine receptor activity ▪ Protein binding ▪ T-cell proliferation ▪ Interleukin-27 receptor binding
ENSSTOG00000028463	MKX	mohawk homeobox		▪ DNA binding ▪ Sequence-specific DNA binding ▪ Regulation of transcription, DNA-templated

				▪ Nucleus
ENSSTOG00000007829	TNNI2	troponin I2, fast skeletal type		▪ Troponin complex ▪ Nucleus ▪ Actin binding ▪ Protein binding ▪ Positive regulation of transcription ▪ DNA-templated ▪ Skeletal muscle contraction ▪ Troponin T binding
ENSSTOG00000020847	Missing	Missing		▪ Thiol-dependent ubiquitinyl hydrolase activity ▪ Protein deubiquitination ▪ Ubiquitin-dependent protein catabolic process ▪ Protein binding ▪ Response to stilbenoid ▪ ISG15-specific protease activity
ENSSTOG00000001180	CD163	CD163 molecule		▪ Integral component of membrane ▪ Membrane ▪ Scavenger receptor activity ▪ Receptor-mediated endocytosis ▪ Protein binding
ENSSTOG00000026716	VCAN	versican		▪ Hyaluronic acid binding ▪ Cell adhesion ▪ Calcium ion binding ▪ Extracellular space ▪ Membrane ▪ Protein binding ▪ Extracellular matrix ▪ Intracellular membrane-bounded organelle ▪ Osteoblast differentiation
ENSSTOG00000020288	ISG15	ISG15 ubiquitin-like modifier		▪ Extracellular region ▪ Defense response to bacterium ▪ Protein binding

				▪ Negative regulation of viral genome replication ▪ Defense response to virus ▪ Negative regulation of protein ubiquitination ▪ ISG15-protein conjugation ▪ Regulation of interferon-gamma production ▪ Response to type I interferon ▪ Protein tag ▪ Modification-dependent protein catabolic process ▪ Positive regulation of erythrocyte differentiation
ENSSTOG00000003422	CAPN9	calpain 9		▪ Intracellular ▪ Digestion ▪ Calcium-dependent cysteine-type endopeptidase activity ▪ Cysteine-type peptidase activity ▪ Peptidase activity ▪ Calcium ion binding ▪ Hydrolase activity ▪ Proteolysis
ENSSTOG00000028862	EEF1A2	eukaryotic translation elongation factor 1 alpha 2	This gene encodes an isoform of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome	▪ GTPase activity ▪ Translational elongation ▪ Translation elongation factor activity ▪ GTP binding ▪ Nucleotide binding ▪ Cytoplasm ▪ Protein binding ▪ Protein kinase binding ▪ Positive regulation of lipid kinase activity ▪ Eukrayotic translation elongation factor 1 complex ▪ Positive regulation of apoptotic process

				▪ Myelin sheath
ENSSTOG00000007570	CLEC4F	C-type lectin domain family 4 member F		▪ Integral component of membrane ▪ Membrane
ENSSTOG00000000900	SERPINA6	serpin family A member 6		▪ Extracellular space ▪ Extracellular exosome ▪ Steroid binding ▪ Glucocorticoid metabolic process
ENSSTOG00000012655	SLCO2B1	solute carrier organic anion transporter family member 2B1		▪ Ion transport ▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Transporter activity
ENSSTOG00000028432	Missing	Missing		▪ Metabolic process ▪ Catalytic activity ▪ Mitochondrion
ENSSTOG00000008000	ALAS2	5'-aminolevulinate synthase 2		▪ Mitochondrion ▪ Heme biosynthetic process ▪ Protoporphyrinogen IX biosynthetic process ▪ Porphyrin-containing compound metabolic process ▪ 5-aminolevulinate synthase activity ▪ Biosynthetic process ▪ Tetrapyrrole biosynthetic process ▪ Transferase activity, transferring acyl groups ▪ Pyridoxal phosphate binding ▪ Metabolic process ▪ Transferase activity ▪ Mitochondrial matrix ▪ Catalytic activity ▪ Mitochondrial inner membrane

				▪ Protein binding ▪ Response to hypoxia ▪ Cellular iron ion homeostasis ▪ Erythrocyte differentiation ▪ Hemoglobin biosynthetic process
ENSSTOG00000012846	MX1	MX dynamin like GTPase 1		▪ GTPase activity ▪ GTP binding ▪ Nucleotide binding ▪ Cytoplasm ▪ Cytosol ▪ Protein binding ▪ Perinuclear region of cytoplasm ▪ Response to virus ▪ Negative regulation of viral genome replication ▪ Nuclear membrane
ENSSTOG00000003382	TNC	tenascin C	It is implicated in guidance of migrating neurons as well as axons during development, synaptic plasticity, and neuronal regeneration.	▪ Extracellular matrix ▪ Cell adhesion ▪ Regulation of cell proliferation ▪ Extracellular space ▪ Membrane ▪ Focal adhesion ▪ Response to wounding ▪ Syndecan binding ▪ Osteoblast differentiation ▪ Extracellular region ▪ Positive regulation of gene expression ▪ Positive regulation of cell proliferation ▪ Basement membrane ▪ Neuromuscular junction development ▪ Interstitital matrix ▪ Prostate gland epithelium morphogenesis ▪ Peripheral nervous system axon regeneration

				▪ Mesenchymal-epithelial cell signaling involved in prostate gland development
ENSSTOG00000022777	Missing	Missing		▪ Iron ion binding ▪ Transport ▪ Metal ion binding ▪ Heme binding ▪ Hemoglobin complex ▪ Oxygen binding ▪ Oxygen transport ▪ Oxygen transporter activity
ENSSTOG00000010129	FABP7	fatty acid binding protein 7		▪ Transport ▪ Lipid binding, transporter activity ▪ Cytoplasm ▪ Nucleoplasm ▪ Cell periphery ▪ Cell-cell junction ▪ Neuronal cell body ▪ Neurogenesis ▪ Startle response ▪ Cell body ▪ Cell projection ▪ Prepulse inhibition ▪ Cell proliferation in forebrain
ENSSTOG00000023860	Missing	Missing		▪ Integral component of membrane ▪ Membrane ▪ Protein binding ▪ Integral component of plasma membrane ▪ Apoptotic process ▪ Cellular response to tumor necrosis factor ▪ Negative regulation of myelination ▪ Myelination ▪ T cell receptor signaling pathway ▪ Axon ▪ Neuron apoptotic process

				• Adaptive immune response • Humoral immune response • Negative regulation of T cell proliferation • Intrinsic component of plasma membrane • B cell apoptotic process • Regulation of oligodendrocyte differentiation • Oligodendrocyte apoptotic process • Negative regulation of B cell proliferation • Negative regulation of interleukin-10 secretion • Negative regulation of interleukin-5 secretion • Negative regulation of interleukin-13 secretion • Signal transduction
ENSSTOG00000006404	PTCH2	patched 2		• Integral component of membrane • Membrane • Hedgehog receptor activity • Signal transduction • Smoothened binding • Hedgehog family protein binding • Epidermis development • Skin development • Positive regulation of epidermal cell differentiation • Hair cycle • Cell fate determination • Epidermal cell fate specification
ENSSTOG00000025073	Missing	Missing		• Iron ion binding • Transport • Metal ion binding • Heme binding • Hemoglobin complex

				▪ Oxygen binding ▪ Oxygen transport ▪ Oxygen transporter activity
ENSSTOG00000024317	Missing	Missing		▪ Iron ion binding ▪ Transport ▪ Metal ion binding ▪ Heme binding ▪ Hemoglobin complex ▪ Oxygen binding ▪ Oxygen transport ▪ Oxygen transporter activity
ENSSTOG00000009418	PLCD3	phospholipase C delta 3		▪ Intracellular ▪ Lipid catabolic process ▪ Phosphoric diester hydrolase activity ▪ Lipid metabolic process ▪ Signal transducer activity ▪ Phosphatidylinositol phospholipase C activity ▪ Regulation of cell proliferation ▪ Signal transduction ▪ Angiogenesis ▪ Intracellular signal transduction ▪ Hydrolase activity ▪ Labyrinthine layer blood vessel development
ENSSTOG00000010414	PALLD	palladin, cytoskeletal associated protein		▪ Integral component of membrane ▪ Membrane ▪ Cell migration ▪ Actin cytoskeleton organization ▪ Actin binding ▪ Cytoplasm ▪ Plasma membrane ▪ Nucleus ▪ Mitochondrion

				▪ Protein binding ▪ Actin cytoskeleton ▪ Actin filament ▪ Focal adhesion ▪ Epithelial cell morphogenesis ▪ Keratinocyte development
ENSSTOG00000010650	Missing	Missing		▪ Iron ion binding ▪ Transport ▪ Metal ion binding ▪ Heme binding ▪ Hemoglobin complex ▪ Oxygen binding ▪ Oxygen transport ▪ Oxygen transporter activity
ENSSTOG00000002545	Missing	Missing		▪ GTPase activity ▪ GTP binding ▪ Nucleotide binding
ENSSTOG00000005711	IRF7	interferon regulatory factor 7		▪ Regulatory region DNA binding ▪ Transcription factor activity, sequence-specific DNA binding ▪ Regulation of transcription, DNA-templated ▪ Cytoplasm ▪ Nucleus ▪ DNA binding ▪ Protein binding ▪ Positive regulation of transcription from RNA polymerase II promoter ▪ Positive regulation of transcription, DNA-templated ▪ Nucleoplasm ▪ Positive regulation of interferon-alpha production ▪ Positive regulation of interferon-beta production

				▪ Transcription factor activity, RNA polymerase II core promoter proximal region sequence-specific binding ▪ RNA polymerase II core promoter sequence-specific DNA binding ▪ Regulation of adaptive immune response ▪ Immunoglobulin mediated immune response ▪ Positive regulation of type I interferon-mediated signaling pathway ▪ Positive regulation of type I interferon production ▪ Regulation of MyD88-dependent toll-like receptor signaling pathway ▪ Type I interferon biosynthetic process ▪ Interferon-alpha production ▪ Interferon-beta production ▪ Regulation of MyD88-independent toll-like receptor signaling pathway
ENSSTOG00000013516	IFI44	interferon induced protein 44		

Table S4.10 - Over expressed genes in NY winter & AK (NYw vs NYs and AK vs NYs)

Gene	Name	Description	Gene card	GO Terms
ENSSTOG00000002629	KDELR3	KDEL endoplasmic reticulum protein retention receptor 3		▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ ER retention sequence binding ▪ Protein retention in ER lumen ▪ Protein transport ▪ Endoplasmic reticulum membrane ▪ Endoplasmic reticulum
ENSSTOG00000006538	ATOH8	atonal bHLH transcription factor 8		▪ Cell differentiation ▪ Transcription factor activity, sequence-specific DNA binding ▪ Protein dimerization activity ▪ Regulation of transcription, DNA templated ▪ Nucleus ▪ Positive regulation of transcription, DNA templated ▪ SMAD protein signal transduction ▪ E-box binding ▪ Negative regulation of endothelial cell proliferation ▪ Postive regulation of endothelial cell migration ▪ Tube formation ▪ Postive regulation of endothelial cell differentiation ▪ Cytoplasm ▪ Transcription factor binding ▪ Negative regulation of transcription, DNA templated

				▪ Activating transcription factor binding ▪ Formation of primary germ layer ▪ Myoblast proliferation
ENSSTOG00000011815	Missing	Missing		▪ Calcium ion binding ▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Cell adhesion ▪ Extracellular exosome ▪ Cytoplasm ▪ Corticospinal tract morphogenesis
ENSSTOG00000020259	Missing	Missing		▪ Hydrolase activity, hydrolyzing O-glycosyl compounds ▪ Carbohydrate metabolic process
ENSSTOG00000021197	Missing	Missing		▪ Calcium ion binding ▪ Cytoplasm ▪ Nucleus ▪ Membrane ▪ Protein binding ▪ Nucleolus ▪ Golgi apparatus ▪ Positive regulation of calcium ion transport ▪ Activation of store-operated calcium channel activity ▪ Store-operated calcium entry
ENSSTOG00000021898	CYP26B1	cytochrome P450 family 26 subfamily B member 1		▪ Iron ion binding ▪ Oxidation-reduction process ▪ Oxidoreductase activity ▪ Metal ion binding ▪ Heme binding ▪ Monooxygenase activity

				▪ Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen ▪ Cytoplasm ▪ Bone morphogenesis Retinoic acid binding ▪ Retinoic acid 4-hydroxylase activity ▪ Retinoic acid catabolic process ▪ Spermatogenesis ▪ Inflammatory response ▪ Positive regulation of gene expression ▪ Negative regulation of retinoic acid receptor signaling pathway ▪ Establishment of skin barrier ▪ Cellular response to retinoic acid ▪ Male meiosis ▪ Retinoic acid receptor signaling pathway ▪ Regulation of T cell differentiation ▪ Cell fate determination ▪ Embryonic limb morphogenesis ▪ Proximal/distal pattern formation ▪ Establishment of T cell polarity ▪ Cornification ▪ Regulation of retinoic acid receptor signaling pathway ▪ Tongue morphogenesis ▪ Positive regulation of tongue muscle cell differentiation
ENSSTOG00000024583	CISH	cytokine inducible SH2 containing protein	The expression of this gene can be induced by IL2, IL3, GM-CSF and EPO in hematopoietic cells .	▪ Intracellular ▪ Protein ubiquitination ▪ Intracellular signal transduction ▪ Protein binding ▪ Plasma membrane

			Proteasome-mediated degradation of this protein has been shown to be involved in the inactivation of the erythropoietin receptors.	▪ Protein kinase C-activating G-protein coupled receptor signaling pathway

Table S4.11 - Over expressed in AK and CO (AK vs NY and CO vs WV)

Gene	Name	Description	Gene Card	GO Terms
ENSSTOG00000001380	RASGRF2	Ras protein specific guanine nucleotide releasing factor 2		▪ Intracellular ▪ Regulation of Ras protein signal transduction ▪ Positive regulation of GTPase activity ▪ Regulation of Rho protein signal transduction ▪ Rho guanyl-nucleotide exchange factor activity ▪ Guanyl-nucleotide exchange factor activity ▪ Small GTPase mediated signal transduction
ENSSTOG00000007918	RBM46	RNA binding motif protein 46		▪ Nucleic acid binding ▪ Nucleotide binding
ENSSTOG00000008371	PIP5KL1	phosphatidylinositol-4-phosphate 5-kinase like 1		▪ Phosphatidylinositol metabolic process ▪ Phosphatidylinositol phosphate kinase activity ▪ Cytoplasm ▪ Protein binding ▪ Cell projection ▪ 1-phosphatidylinositol-4-phosphate 5-kinase activity
ENSSTOG00000011815	Missing	Missing		▪ Calcium ion binding ▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Cell adhesion ▪ Extracellular exosome ▪ Cytoplasm

				▪ Corticospinal tract morphogenesis
ENSSTOG00000012025	LBP	lipopolysaccharide binding protein		▪ Defense response to Gram-negative bacterium ▪ Lipopolysaccharide binding ▪ Lipid binding ▪ Innate immune response ▪ Extracellular space ▪ Acute-phase response ▪ Protein binding ▪ Extracellular exosome ▪ Response to lipopolysaccharide ▪ Cell surface ▪ Cellular response to lipopolysaccharide ▪ Negative regulation of tumor necrosis factor production ▪ Lipopolysaccharide-mediated signaling pathway ▪ Positive regulation of interleukin-6 production ▪ Positive regulation of interleukin-8 production ▪ Positive regulation of tumor necrosis factor production ▪ Defense response to Gram-positive bacterium ▪ Cellular response to lipoteichoic acid ▪ Positive regulation of macrophage activation ▪ Positive regulation of toll-like receptor 4 signaling pathway ▪ Lipoteichoic acid binding ▪ Detection of molecule of bacterial origin ▪ Macrophage activation involved in immune response

				▪ Lipopolysaccharide transport ▪ Receptor binding ▪ Positive regulation of chemokine production ▪ Positive regulation of neutrophil chemotaxis ▪ Negative regulation of growth of symbiont in host ▪ Positive regulation of tumor necrosis factor biosynthetic process ▪ Leukocyte chemotaxis involved in inflammatory response ▪ Positive regulation of respiratory burst involved in inflammatory response
ENSSTOG00000020720	WFDC2	WAP four-disulfide core domain 2		▪ Peptidase inhibitor activity ▪ Extracellular region ▪ Extracellular exosome ▪ Serine-type endopeptidase inhibitor activity ▪ Aspartic-type endopeptidase inhibitor activity
ENSSTOG00000021630	Missing	Missing		▪ Oxidation-reduction process ▪ Oxidoreductase activity ▪ 3-beta-hydroxy-delta5-steroid dehydrogenase activity ▪ Steroid biosynthetic process ▪ Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor
ENSSTOG00000023922	SCARA3	scavenger receptor class A member 3		▪ Integral component of membrane ▪ Membrane ▪ Protein binding

ENSSTOG00000024724	SLC16A11	solute carrier family 16 member 11	Probably involved in hepatic lipid metabolism: overexpression results in an increase of triacylglycerol(TAG) levels, small increases in intracellular diacylglycerols and decreases in lysophosphatidylcholine, cholesterol ester and sphingomyelin lipids	- Integral component of membrane - Membrane - Transmembrane transport - Endoplasmic reticulum membrane - Lipid metabolic process
ENSSTOG00000026668	LAMA5	laminin subunit alpha 5		- Regulation of embryonic development - Regulation of cell adhesion - Regulation of cell migration - Receptor binding - Cell adhesion - Nucleus - Extracellular space - Extracellular exosome - Extracellular matrix - Basement membrane - Integrin-mediated signaling pathway - Cell migration - Integrin binding - Substrate adhesion-dependent cell spreading - Laminin-10 complex - Protein binding - Proteinaceous extracellular matrix - Regulation of cell proliferation - Cilium assembly - Muscle organ development - Odontogenesis of dentin-containing tooth

				▪ Morphogenesis of embryonic epithelium ▪ Hair follicle development ▪ Basal lamina ▪ Organ morphogenesis ▪ Lung development ▪ Establishment of protein localization to plasma membrane ▪ Branching involved in ureteric bud morphogenesis ▪ Branching morphogenesis of an epithelial tube ▪ Neural crest cell migration ▪ Branching involved in salivary gland morphogenesis ▪ Morphogenesis of a polarized epithelium
ENSSTOG00000026865	SHC2	SHC adaptor protein 2		▪ Intracellular ▪ Activation of MAPK activity ▪ Receptor tyrosine kinase binding ▪ Intracellular signal transduction ▪ Protein binding
ENSSTOG00000028017	CHRNA4	cholinergic receptor nicotinic alpha 4 subunit		▪ Ion transport ▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Extracellular ligand-gated ion channel activity ▪ Acetylcholine-activated cation-selective channel activity ▪ Postsynaptic membrane ▪ Synapse ▪ Cell junction ▪ Ion channel activity ▪ Signal transduction

				Response to oxidative stress DNA repair Acetylcholine receptor activity Response to hypoxia Cognition Neurological system process Acetylcholine-gated channel complex Response to nicotine Behavioral response to nicotine External side of plasma membrane Protein binding B cell activation Action potential Regulation of membrane potential Calcium ion transport Locomotory behavior Synaptic transmission, cholinergic Respiratory gaseous exchange Regulation of dopamine secretion Sensory perception of pain Exploration behavior Membrane depolarization Inhibitory postsynaptic potential

Table S4.12 - Over expressed in CO & NY (CO vs WV and NY vs WV)

Gene	Name	Description	Gene Card	GO Terms
ENSSTOG00000000004	MYO3A	myosin IIIA		▪ Myosin complex ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Actin binding ▪ Motor activity ▪ Nucleotide binding ▪ Filopodium ▪ Sensory perception of sound ▪ Calmodulin binding ▪ Filamentous actin ▪ Protein autophosphorylation ▪ ADP binding ▪ Microfilament motor activity ▪ Actin-dependent ATPase activity ▪ Plus-end directed microfilament motor activity
ENSSTOG00000000314	Missing	Missing		▪ Integral component of membrane ▪ Membrane ▪ Transmembrane transport ▪ Sulfate transmembrane transport ▪ Basolateral plasma membrane ▪ Sulfate transport ▪ Secondary active sulfate transmembrane transporter activity ▪ Gastric acid secretion ▪ Anion transmembrane transport ▪ Anion transmembrane transporter activity ▪ Anion transport

ENSSTOG00000000900	SERPINA6	serpin family A member 6		▪ Extracellular space ▪ Extracellular exosome ▪ Steroid binding ▪ Glucocorticoid metabolic process
ENSSTOG00000002740	GCK	glucokinase		▪ Carbohydrate phosphorylation ▪ Phosphotransferase activity, alcohol group as acceptor ▪ Glycolytic process ▪ Glucose binding ▪ Hexokinase activity ▪ Cellular glucose homeostasis ▪ Transferase activity ▪ Phosphorylation ▪ Kinase activity ▪ Carbohydrate metabolic process ▪ ATP binding ▪ Nucleotide binding ▪ Protein binding ▪ Negative regulation of gluconeogenesis ▪ Positive regulation of insulin secretion ▪ Regulation of insulin secretion ▪ Glucose homeostasis ▪ Positive regulation of glycogen biosynthetic process ▪ Glucokinase activity ▪ Detection of glucose ▪ Nucleus ▪ Mitochondrion ▪ Cytosol ▪ Glucose metabolic process ▪ Calcium ion import ▪ Regulation of potassium ion transport ▪ NADP metabolic process

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ENSSTOG00000003422	CAPN9	calpain 9		- Intracellular - Digestion - Calcium-dependent cysteine-type endopeptidase activity - Cysteine-type peptidase activity - Peptidase activity - Calcium ion binding - Hydrolase activity - Proteolysis
ENSSTOG00000006286	TNFSF10	tumor necrosis factor superfamily member 10		- Membrane - Tumor necrosis factor receptor binding - Immune response - Cytokine activity - Extracellular space - Protein binding - Extracellular exosome - Positive regulation of I-kappaB kinase/NF-kappaB signaling - Positive regulation of apoptotic process - Positive regulation of release of cytochrome c from mitochondria - Positive regulation of cysteine-type endopeptidase activity involved in apoptotic process - Positive regulation of extrinsic apoptotic signaling pathway - Tumor necrosis factor receptor superfamily binding
ENSSTOG00000007004	Missing	Missing		- Integral component of membrane - Membrane - Plasma membrane - Protein binding - Cell-matrix adhesion - Cell surface

ENSSTOG00000009959	FLNB	filamin B		- Actin cytoskeleton organization - Cell differentiation - Actin binding - Cytoplasm - Plasma membrane - Poly(A) RNA binding - Protein binding - Identical protein binding - Extracellular exosome - Focal adhesion - Epithelial cell morphogenesis - Keratinocyte development - Stress fiber - Brush border - Skeletal muscle tissue development
ENSSTOG00000010036	COL27A1	collagen type XXVII alpha 1		- Proteinaceous extracellular matrix - Collagen trimer - Extracellular matrix structural constituent - Extracellular matrix organization - Fibrillar collagen trimer - Growth plate cartilage chondrocyte development
ENSSTOG00000010923	CYP7B1	cytochrome P450 family 7 subfamily B member 1	The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum membrane protein catalyzes the first reaction in the cholesterol catabolic	- Iron ion binding - Oxidation-reduction process - Oxidoreductase activity - Integral component of membrane - Membrane - Metal ion binding - Heme binding - Monooxygenase activity - Endoplastic reticulum membrane - Endoplasmic reticulum

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		pathway of extrahepatic tissues, which converts cholesterol to bile acids. This enzyme likely plays a minor role in total bile acid synthesis, but may also be involved in the development of atherosclerosis, neurosteroid metabolism and sex hormone synthesis		- Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen - Positive regulation of epithelial cell proliferation - Bile acid biosynthetic process - Prostate gland epithelium morphogenesis - Negative regulation of intracellular estrogen receptor signaling pathway - Oxysterol 7-alpha-hydroxylase activity
ENSSTOG00000011601	Missing	Missing		- Ion transport - Transport - Integral component of membrane - Membrane - Transmembrane transport - Regulation of postsynaptic membrane potential - Sodium ion transmembrane transport - Sodium ion transport - Sodium channel activity - Voltage-gated sodium channel activity - Voltage-gated sodium channel complex - Ion transmembrane transport - Regulation of ion transmembrane transport - Voltage-gated ion channel activity - Ion channel activity
ENSSTOG00000019919	Missing	Missing		- Glutathione transferase activity - Metabolic process
ENSSTOG00000023978	OSGIN1	oxidative stress induced growth inhibitor 1		- Oxidation-reduction process - Oxidoreductase activity - Positive regulation of apoptotic process - Negative regulation of cell growth

				▪ Protein binding ▪ Growth factor activity
ENSSTOG00000024039	KLHDC7B	kelch domain containing 7B		
ENSSTOG00000024424	Missing	Missing		▪ Exocytosis ▪ Vesicle docking ▪ Cytoplasm ▪ Ral GTPase binding ▪ Protein N-terminus binding
ENSSTOG00000026191	ANGPTL8	angiopoietin like 8		▪ Extracellular region ▪ Protein binding ▪ Regulation of lipid metabolic process ▪ Triglyceride homeostasis ▪ Positive regulation of protein processing ▪ Regulation of lipoprotein metabolic process ▪ Hormone activity ▪ Fat cell differentiation ▪ Cell maturation ▪ Cellular lipid metabolic process ▪ Type B pancreatic cell proliferation
ENSSTOG00000027194	MID1IP1	MID1 interacting protein 1		▪ Protein binding ▪ Cytoplasm ▪ Nucleus ▪ Microtubule cytoskeleton ▪ Cytosol ▪ Protein C-terminus binding ▪ Protein polymerization ▪ Negative regulation of microtubule depolymerization ▪ Positive regulation of fatty acid biosynthetic process ▪ Positive regulation of ligase activity

ENSSTOG00000027330	MUC5AC	mucin 5AC, oligomeric mucus/gel-forming		- Extracellular region - Cytoplasm - Extracellular space - Extracellular exosome - Fibril - Phosphatidylinositol-mediated signaling - Extracellular fibril organization
ENSSTOG00000027918	Missing	Missing		- Integral component of membrane - Membrane - Plasma membrane - Homophilic cell adhesion via plasma membrane adhesion molecules - Cell adhesion - Calcium ion binding
ENSSTOG00000027993	LONRF1	LON peptidase N-terminal domain and ring finger 1		- Metal ion binding - ATP-dependent peptidase activity - Zinc ion binding - Proteolysis - Ubiquitin-protein transferase activity - Protein binding
ENSSTOG00000028594	Missing	Missing		- Signal transduction - Protein binding - Axon extension - Cell leading edge

Table S4.13 - Over expressed in NY & MT (NY vs WV and MT vs NY)

Gene	Name	Description	Gene Card	GO Terms
ENSSTOG00000010923	CYP7B1	cytochrome P450 family 7 subfamily B member 1	The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum membrane protein catalyzes the first reaction in the cholesterol catabolic pathway of extrahepatic tissues, which converts cholesterol to bile acids. This enzyme likely plays a minor role in total bile acid synthesis, but may also be involved in the development of atherosclerosis, neurosteroid metabolism and sex hormone synthesis	- Iron ion binding - Oxidation-reduction process - Oxidoreductase activity - Integral component of membrane - Membrane - Metal ion binding - Heme binding - Monooxygenase activity - Endoplastic reticulum membrane - Endoplasmic reticulum - Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen - Positive regulation of epithelial cell proliferation - Bile acid biosynthetic process - Prostate gland epithelium morphogenesis - Negative regulation of intracellular estrogen receptor signaling pathway - Oxysterol 7-alpha-hydroxylase activity

Table S4.14 - Over expressed in NY & NY (NYs vs NYw and NYs vs AK)

Gene	Name	Description	Gene Card	GO Term
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development. Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation.	▪ Membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Peptidyl-tyrosine phosphorylation ▪ Protein tyrosine kinase activity ▪ Calcium ion binding ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Cytoplasm ▪ Plasma membrane ▪ Protein binding ▪ Integral component of plasma membrane ▪ Receptor complex ▪ Endosome membrane ▪ Intracellular membrane-bounded organelle ▪ Positive regulation of cell migration ▪ Regulation of cell adhesion ▪ Positive regulation of cell adhesion mediated by integrin ▪ Cellular response to reinoic acid ▪ Neuron cell-cell adhesion ▪ Positive regulation of neuron projection development ▪ Membrane protein proteolysis ▪ MAPK cascade ▪ Positive regulation of transcription, DNA templated ▪ Positive regulation of gene expression ▪ Transmembrane receptor protein tyrosine kinase signaling pathway

				▪ Nervous system development ▪ Positive regulation of cell size ▪ Neuron differentiation ▪ Regulation of axonogenesis ▪ Ureteric bud development ▪ Positive regulation of serine phophoylation of STAT3 protein ▪ Neural crest cell migration ▪ Enteric nervous system development ▪ Response to pain ▪ Neuron maturation ▪ Embryonic epithelial tube formation ▪ Ureter maturation ▪ Positive regulation of metanephric glomerulus development ▪ Peyer's patch morphogenesis
ENSSTOG00000004459	Missing	Missing		▪ Integral component of membrane
ENSSTOG00000007820	Missing	Missing		▪ Integral component of membrane ▪ Membrane ▪ Carbohydrate binding ▪ Golgi membrane ▪ Transferase activity, transferring glycosyl groups ▪ Protein glycosylation ▪ Golgi apparatus ▪ Transferase activity
ENSSTOG00000009418	PLCD3	phospholipase C delta 3		▪ Intracellular ▪ Lipid catabolic process ▪ Phosphoric diester hydrolase activity ▪ Lipid metabolic process ▪ Signal transducer activity ▪ Phosphatidylinositol phospholipase C activity

				▪ Regulation of cell proliferation ▪ Signal transduction ▪ Angiogenesis ▪ Intracellular signal transduction ▪ Hydrolase activity ▪ Labyrinthine layer blood vessel development
ENSSTOG00000025236	CFAP100	cilia and flagella associated protein 100		
ENSSTOG00000028432	Missing	Missing		▪ Metabolic process ▪ Catalytic activity ▪ Mitochondrion

Table S4.15 - Over expressed genes in NY & WV (NY vs AK and WV vs CO)

Gene	Name	Description	Gene Card	GO Terms
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development. Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation.	▪ Membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Peptidyl-tyrosine phosphorylation ▪ Protein tyrosine kinase activity ▪ Calcium ion binding ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Cytoplasm ▪ Plasma membrane ▪ Protein binding ▪ Integral component of plasma membrane ▪ Receptor complex ▪ Endosome membrane ▪ Intracellular membrane-bounded organelle ▪ Positive regulation of cell migration ▪ Regulation of cell adhesion ▪ Positive regulation of cell adhesion mediated by integrin ▪ Cellular response to reinoic acid ▪ Neuron cell-cell adhesion ▪ Positive regulation of neuron projection development ▪ Membrane protein proteolysis ▪ MAPK cascade ▪ Positive regulation of transcription, DNA templated ▪ Positive regulation of gene expression ▪ Transmembrane receptor protein tyrosine kinase signaling pathway

				▪ Nervous system development ▪ Positive regulation of cell size ▪ Neuron differentiation ▪ Regulation of axonogenesis ▪ Ureteric bud development ▪ Positive regulation of serine phophoylation of STAT3 protein ▪ Neural crest cell migration ▪ Enteric nervous system development ▪ Response to pain ▪ Neuron maturation ▪ Embryonic epithelial tube formation ▪ Ureter maturation ▪ Positive regulation of metanephric glomerulus development ▪ Peyer's patch morphogenesis
ENSSTOG00000004459	Missing	Missing		▪ Integral component of membrane
ENSSTOG00000004955	SLC30A2	Solute carrier family 30 member 2		▪ Integral component of membrane ▪ Membrane ▪ Transmembrane transport ▪ Cation transmembrane transporter activity ▪ Cation transmembrane transport ▪ Cation transport ▪ Cytoplasm ▪ Protein binding ▪ Late endosome ▪ Lysosomal membrane ▪ Positive regulation of sequestering of zinc ion
ENSSTOG00000004982	TRIM63	tripartite motif containing 63	This protein plays an important role in the atrophy of skeletal and cardiac muscle and is	▪ Intracellular ▪ Metal ion binding ▪ Zinc ion binding ▪ Cytoplasm

			required for the degradation of myosin heavy chain proteins, myosin light chain, myosin binding protein, and for muscle-type creatine kinases.	- Protein binding - Titin binding
ENSSTOG00000008679	CRISPLD1	cysteine rich secretory protein LCCL domain containing 1		- Extracellular region - Extracellular exosome - Face morphogenesis
ENSSTOG00000020175	Missing	Missing		- Integral component of membrane - Membrane - Scavenger receptor activity - Receptor-mediated endocytosis
ENSSTOG00000021636	PPP1R3G	protein phosphatase 1 regulatory subunit 3G	Glycogen-targeting subunit for protein phosphatase 1 (PP1). Involved in the regulation of hepatic glycogenesis in a manner coupled to the fasting-feeding cycle and distinct from other glycogen-targeting subunits	- Cytoplasm - Glucose homeostasis - Protein phosphatase binding - Positive regulation of glycogen biosynthetic process - Positive regulation of glycogen (starch) synthase activity - Glycogen binding
ENSSTOG00000022426	TSPAN32	tetraspanin 32	This gene, which is a member of the tetraspanin superfamily, is one of several tumor-suppressing subtransferable fragments located in the imprinted gene domain of chromosome 11p15.5, an	- Integral component of membrane - Membrane - Intracellular - Cell surface - Integrin-mediated signaling pathway - Cytoskeleton organization - Negative regulation of cell proliferation - Regulation of defense response to virus - Defense response to protozoan

			important tumor-suppressor gene region.	• Blood coagulation • Platelet aggregation • Hemostasis • Negative regulation of myeloid dendritic cell activation • Integrin alphaIIb-beta3 complex
ENSSTOG00000022668	EVPL	envoplakin	Component of the cornified envelope of keratinocytes. May link the cornified envelope to desmosomes and intermediate filaments. Gene Wiki entry for EVPL Gene	• Intermediate filament binding • Epidermis development • Cornified envelope • Cytoskeleton • Structural molecule activity • Cytoplasm • Extracellular exosome • Protein binding • Peptide cross-linking • Keratinocyte differentiation • Intermediate filament cytoskeleton
ENSSTOG00000026217	SACS	sacsin molecular chaperone	Co-chaperone which acts as a regulator of the Hsp70 chaperone machinery and may be involved in the processing of other ataxia-linked proteins.	• Cytoplasm • Nucleus • Mitochondrion • Proteasome binding • Chaperone binding • Hsp70 protein binding • Negative regulation of inclusion body assembly
ENSSTOG00000027573	COMT	catechol-O-methyltransferase	Catalyzes the O-methylation, and thereby the inactivation, of catecholamine neurotransmitters and catechol hormones. Also shortens the biological half-lives of certain neuroactive drugs, like L-DOPA, alpha-	• Integral component of membrane • Membrane • Neurotransmitter catabolic process • Catechol O-methyltransferase activity • Catecholamine metabolic process • O-methyltransferase activity • Methylation • Magensium ion binding • Protein binding

			methyl DOPA and isoproterenol.	▪ Extracellular exosome ▪ Mitochondrion ▪ Dopamine metabolic process ▪ Catechol-containing compound metabolic process ▪ Cellular response to phosphate starvation ▪ Dopamine catabolic process
ENSSTOG00000027956	Missing	Missing		▪ Mitochondrion ▪ Glycine N-acyltransferase activity
ENSSTOG00000028432	Missing	Missing		▪ Metabolic process ▪ Catalytic activity ▪ Mitochondrion

Table S4.16 - Over expressed genes in NY & WV (NY vs NYw and WV vs CO)

Gene	Name	Description	Gene Card	GO Terms
ENSSTOG00000001180	CD163	CD163 molecule		▪ Integral component of membrane ▪ Membrane ▪ Scavenger receptor activity ▪ Receptor-mediated endocytosis ▪ Protein binding
ENSSTOG00000001482	RET	ret proto-oncogene	Plays crucial role in neural crest development. Is a member of the cadherin superfamily, encodes one of the receptor tyrosine kinases, which are cell-surface molecules that transduce signals for cell growth and differentiation.	▪ Membrane ▪ Homophilic cell adhesion via plasma membrane adhesion molecules ▪ Peptidyl-tyrosine phosphorylation ▪ Protein tyrosine kinase activity ▪ Calcium ion binding ▪ Protein phosphorylation ▪ ATP binding ▪ Protein kinase activity ▪ Cytoplasm ▪ Plasma membrane ▪ Protein binding ▪ Integral component of plasma membrane ▪ Receptor complex ▪ Endosome membrane ▪ Intracellular membrane-bounded organelle ▪ Positive regulation of cell migration ▪ Regulation of cell adhesion ▪ Positive regulation of cell adhesion mediated by integrin ▪ Cellular response to reinoic acid ▪ Neuron cell-cell adhesion ▪ Positive regulation of neuron projection development ▪ Membrane protein proteolysis

				▪ MAPK cascade ▪ Positive regulation of transcription, DNA templated ▪ Positive regulation of gene expression ▪ Transmembrane receptor protein tyrosine kinase signaling pathway ▪ Nervous system development ▪ Positive regulation of cell size ▪ Neuron differentiation ▪ Regulation of axonogenesis ▪ Ureteric bud development ▪ Positive regulation of serine phosphorylation of STAT3 protein ▪ Neural crest cell migration ▪ Enteric nervous system development ▪ Response to pain ▪ Neuron maturation ▪ Embryonic epithelial tube formation ▪ Ureter maturation ▪ Positive regulation of metanephric glomerulus development ▪ Peyer's patch morphogenesis
ENSSTOG00000004459	Missing	Missing		▪ Integral component of membrane
ENSSTOG00000006404	PTCH2	patched 2		▪ Integral component of membrane ▪ Membrane ▪ Hedgehog receptor activity ▪ Signal transduction ▪ Smoothened binding ▪ Hedgehog family protein binding ▪ Epidermis development ▪ Skin development ▪ Positive regulation of epidermal cell differentiation

				▪ Hair cycle ▪ Cell fate determination ▪ Epidermal cell fate specification
ENSSTOG00000020288	ISG15	ISG15 ubiquitin-like modifier		▪ Extracellular region ▪ Defense response to bacterium ▪ Protein binding ▪ Negative regulation of viral genome replication ▪ Defense response to virus ▪ Negative regulation of protein ubiquitination ▪ ISG15-protein conjugation ▪ Regulation of interferon-gamma production ▪ Response to type I interferon ▪ Protein tag ▪ Modification-dependent protein catabolic process ▪ Positive regulation of erythrocyte differentiation
ENSSTOG00000026716	VCAN	versican		▪ Hyaluronic acid binding ▪ Cell adhesion ▪ Calcium ion binding ▪ Extracellular space ▪ Membrane ▪ Protein binding ▪ Extracellular matrix ▪ Intracellular membrane-bounded organelle ▪ Osteoblast differentiation
ENSSTOG00000028432	Missing	Missing		▪ Metabolic process ▪ Catalytic activity ▪ Mitochondrion

Table S4.17 - Over expressed genes in WV & NY (WV vs NY and NY vs MT)

Gene	Name	Description	Gene Cards	GO Terms
ENSSTOG00000020350	EIF4EBP3	eukaryotic translation initiation factor 4E binding protein 3	This gene encodes a member of the EIF4EBP family, which consists of proteins that bind to eukaryotic translation initiation factor 4E and regulate its assembly into EIF4F, the multi-subunit translation initiation factor that recognizes the mRNA cap structure.	▪ Negative regulation of translational initiation ▪ Eukaryotic initiation factor 4E binding ▪ Membrane ▪ Protein binding

Table S4.18 - Over expressed genes in WV & WV (WV vs NY and WV vs CO)

Gene	Name	Description	Gene Card	GO Terms
ENSSTOG00000000695	DDIT4	DNA damage inducible transcript 4		▪ Negative regulation of signal transduction ▪ Cytoplasm ▪ Intracellular ▪ Response to hypoxia ▪ Negative regulation of TOR signaling ▪ Mitochondrion ▪ Reactive oxygen species metabolic process ▪ Intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator ▪ 14-3-3 protein binding ▪ Negative regulation of ▪ Glycolytic process ▪ Protein complex disassembly
ENSSTOG00000000928	SEMA4D	semaphorin 4D		▪ Integral component of membrane ▪ Membrane ▪ Positive regulation of GTPase activity ▪ Negative regulation of transcription from RNA polymerase II promoter ▪ Protein binding ▪ Integral component of plasma membrane ▪ Positive regulation of protein phosphorylation ▪ Receptor binding ▪ Transmembrane signaling receptor activity ▪ Receptor activity ▪ Positive regulation of cell migration ▪ Regulation of cell shape

				▪ Positive regulation of phosphatidylinositol 3-kinase signaling ▪ Negative regulation of cell adhesion ▪ Semaphorin-plexin signaling pathway ▪ Semaphorin receptor binding ▪ Ossification involved in bone maturation ▪ Regulation of dendrite morphogenesis ▪ Positive regulation of collateral sprouting ▪ Negative regulation of alkaline phosphatase activity ▪ Regulation of cell projection organization ▪ Leukocyte aggregation ▪ Extracellular space ▪ Positive regulation of peptidyl-tyrosine phosphorylation ▪ Positive regulation of axonogenesis ▪ Negative regulation of osteoblast differentiation ▪ Negative regulation of peptidyl-tyrosine phosphorylation ▪ Semaphorin-plexin signaling pathway involved in bone trabecula morphogenesis
ENSSTOG00000001668	ARPC1B	actin related protein 2/3 complex subunit 1B		▪ Regulation of actin filament polymerization ▪ Actin cytoskeleton ▪ Arp2/3 complex-mediated actin nucleation ▪ Arp2/3 protein complex ▪ Structural constituent of cytoskeleton ▪ Actin filament binding ▪ Extracellular exosome ▪ Focal adhesion

ENSSTOG00000002137	CIDEC	cell death inducing DFFA like effector c		▪ Intracellular ▪ Apoptotic process
ENSSTOG00000003534	MEDAG	mesenteric estrogen dependent adipogenesis		▪ Cytoplasm ▪ Positive regulation of fat cell differentiation
ENSSTOG00000003590	ROPN1L	rhophilin associated tail protein 1 like		▪ Cytoplasm ▪ Protein binding ▪ Motile cilium
ENSSTOG00000003699	ACP5	acid phosphatase 5, tartrate resistant		▪ Acid phosphatase activity ▪ Hydrolase activity ▪ Extracellular exosome ▪ Ferrous iron binding ▪ Ferric iron binding ▪ Defense response to Gram-positive bacterium ▪ Response to lipopolysaccharide ▪ Dephosphorylation ▪ Lysosome ▪ Response to cytokine ▪ Bone morphogenesis ▪ Negative regulation of inflammatory response ▪ Negative regulation of interleukin-12 production ▪ Negative regulation of nitric oxide biosynthetic process ▪ Bone resorption ▪ Negative regulation of tumor necrosis factor production ▪ Negative regulation of interleukin-1 beta production

				▪ Negative regulation of superoxide anion generation
ENSSTOG00000006055	C1orf54	chromosome 1 open reading frame 54		
ENSSTOG00000006335	BPIFB4	BPI fold containing family B member 4		▪ Lipid binding
ENSSTOG00000006538	ATOH8	atonal bHLH transcription factor 8		▪ Cell differentiation ▪ Transcription factor activity, sequence-specific DNA binding ▪ Protein dimerization activity Regulation of transcription, DNA templated ▪ Nucleus ▪ Positive regulation of transcription, DNA templated ▪ SMAD protein signal transduction ▪ E-box binding ▪ Negative regulation of endothelial cell proliferation ▪ Postive regulation of endothelial cell migration ▪ Tube formation ▪ Postive regulation of endothelial cell differentiation ▪ Cytoplasm ▪ Transcription factor binding ▪ Negative regulation of transcription, DNA templated ▪ Activating transcription factor binding ▪ Formation of primary germ layer ▪ Myoblast proliferation

ENSSTOG00000008554	Fabp4	Fatty acid-binding protein, adipocyte		▪ Transport ▪ Hibernation ▪ Regulation of inflammatory response ▪ Fatty acid binding ▪ Lipid binding ▪ Transporter activity ▪ Cytoplasm ▪ Nucleus ▪ Extracellular exosome ▪ Negative regulation of transcription, DNA-templated ▪ Cholesterol homeostasis ▪ White fat cell differentiation ▪ Brown fat cell differentiation ▪ Cytokine production ▪ Negative regulation of protein kinase activity ▪ Positive regulation of inflammatory response ▪ Cellular response to lithium ion
ENSSTOG00000011283	KLHDC8A	kelch domain containing 8A		
ENSSTOG00000019839	GABRR3	gamma-aminobutyric acid type A receptor rho3 subunit (gene/pseudogene)		▪ Ion transport ▪ Transport ▪ Integral component of membrane ▪ Membrane ▪ Plasma membrane ▪ Extracellular ligand-gated ion channel activity ▪ Synapse ▪ Ion transmembrane transport ▪ Cell junction

ENSSTOG00000020350	EIF4EBP3	eukaryotic translation initiation factor 4E binding protein 3	This gene encodes a member of the EIF4EBP family, which consists of proteins that bind to eukaryotic translation initiation factor 4E and regulate its assembly into EIF4F, the multi-subunit translation initiation factor that recognizes the mRNA cap structure.	▪ Negative regulation of translational initiation ▪ Eukaryotic initiation factor 4E binding ▪ Membrane ▪ Protein binding
ENSSTOG00000021850	Missing	Missing		▪ Lysozyme activity
ENSSTOG00000022135	S100A4	S100 calcium binding protein A4		▪ Metal ion binding ▪ Calcium ion binding ▪ Nucleus ▪ Extracellular space ▪ Poly(A) RNA binding ▪ Protein binding ▪ Perinuclear region of cytoplasm ▪ Extracellular exosome ▪ Positive regulation of I-kappaB kinase/NF-kappaB signaling ▪ RAGE receptor binding ▪ Identical protein binding
ENSSTOG00000023060	Missing	Missing		▪ Lysozyme activity
ENSSTOG00000023809	POPDC2	popeye domain containing 2		▪ Integral component of membrane ▪ Membrane ▪ Sarcolemma
ENSSTOG00000024173	FAM134B	family with sequence similarity 134 member B		▪ Integral component of membrane ▪ Membrane ▪ Reticulophagy ▪ Integral component of endoplasmic reticulum membrane

				▪ Protein binding ▪ Negative regulation of neuron apoptotic process ▪ Sensory perception of pain ▪ cis-Golgi network
ENSSTOG00000025781	PPP1R3C	protein phosphatase 1 regulatory subunit 3C		▪ Protein phosphatase regulator activity ▪ Glycogen metabolic process ▪ Regulation of catalytic activity ▪ Carbohydrate metabolic process ▪ Protein binding ▪ Glycogen biosynthetic process ▪ Protein phosphatase binding
ENSSTOG00000027370	GADD45G	growth arrest and DNA damage inducible gamma		▪ Response to stress ▪ Regulation of cell cycle ▪ Nucleus ▪ Cytoplasm Protein binding ▪ Postive regulation of apoptotic process ▪ Positive regulation of JNK cascade ▪ Activation of MAPKKK activity ▪ Positive regulation of p38MAPK cascade
ENSSTOG00000028838	FAM166B	family with sequence similarity 166 member B		