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# Contemporary range expansion of the Virginia opossum (*Didelphis virginiana*) impacted by humans and snow cover

L.L. Walsh and P.K. Tucker

**Abstract:** Range expansions are key demographic events driven by factors such as climate change and human intervention that ultimately influence the genetic composition of peripheral populations. The expansion of the Virginia opossum (*Didelphis virginiana* Kerr, 1792) into Michigan has been documented over the past 200 years, indicating relatively new colonizations in northern Michigan. Although most contemporary expansions are a result of shifts in climate regimes, the opossum has spread beyond its hypothesized climate niche, offering an opportunity to examine the compounding influence that climate change and humans have on a species' distribution. The genetic consequences of two range expansions were investigated using genotypic data for nine microsatellite markers from opossums collected in Michigan, Ohio, and Wisconsin, USA. Two genetic clusters were identified: one on either side of Lake Michigan. Using general linear models, we found that measurements of genetic diversity across 15 counties are best explained by days of snow on the ground. Next best models incorporate anthropogenic covariates including farm density. These models suggest that opossum expansion may be facilitated by agricultural land development and at the same time be limited by their inability to forage in snow.

**Key words:** agriculture, climate change, *Didelphis virginiana*, land-use change, Virginia opossum, population genetics, range expansion.

**Résumé :** Les expansions d'aires de répartition constituent d'importants événements démographiques modulés par des facteurs tels que les changements climatiques et l'intervention humaine et, à terme, influencent la composition génétique de populations périphériques. La documentation de l'expansion de l'opossum de Virginie (*Didelphis virginiana* Kerr, 1792) au Michigan au cours des 200 dernières années indique des colonisations relativement récentes dans le nord de l'État. Si la plupart des expansions récentes sont le résultat de changements de régimes climatiques, l'opossum s'est propagé au-delà de sa niche climatique présumée, offrant du coup l'occasion d'examiner l'influence cumulative des changements climatiques et des humains sur la répartition d'une espèce. Les conséquences génétiques de deux expansions de l'aire de répartition ont été étudiées en utilisant des données génotypiques pour neuf marqueurs microsatellites d'opossums prélevés au Michigan, en Ohio et au Wisconsin (États-Unis). Deux groupements génétiques ont été décelés, de part et d'autre du lac Michigan. En utilisant des modèles linéaires généraux, nous avons constaté que le nombre de jours de neige au sol explique le mieux les mesures de la diversité génétique à l'échelle de 15 comtés. Les meilleurs modèles suivants incorporent des covariables associées à l'activité humaine, dont la densité des fermes. Ces modèles portent à croire que l'expansion des opossums pourrait être facilitée par l'aménagement agricole du territoire et, en même temps, limitée par l'incapacité des opossums de trouver leur nourriture sous la neige. [Traduit par la Rédaction]

**Mots-clés :** agriculture, changements climatiques, *Didelphis virginiana*, changement de l'occupation du territoire, opossum de Virginie, génétique des populations, expansion de l'aire de répartition.

## Introduction

Dynamic range margins can strongly impact the natural history and genetics of a species, making range expansions important systems in both ecology and evolution (Excoffier et al. 2009). At the fronts of range expansions, scientists have discovered evidence of selection for traits that are adaptive to expansion. These traits include dispersal capacity, ability to colonize, and traits directly related to coping with the novel environment (Buckley et al. 2012; Bridle et al. 2014). The genetic diversity of a species at the edge of its range can allow for estimates of how well organisms might adapt to novel environments (Dawson et al. 2010; Buckley et al. 2012). The ability of an expanding species to adapt will determine the magnitude of its effect on a new community and its capability of displacing similar organisms (reviewed by

Eckert et al. 2008). As species expand into novel habitats, they have the potential to significantly alter community composition through competition, predation, and introduction of new parasites. Understanding the population genetic patterns of a range expansion, therefore, is especially important for organisms that are pollinators, pathogens, vectors for disease, scavengers, or generalists that may outcompete specialists and affect the new habitats they encounter (Olson et al. 2012).

The most common cause cited for recent range expansions is climate change, an ever-growing issue that has altered species' ranges throughout the world (Parmesan et al. 2005). General patterns in the Northern Hemisphere indicate northern and high-altitude organisms are losing habitat, whereas southern and low-altitude organisms are experiencing range expansions (Moritz et al. 2008; Myers et al. 2009). To better understand the influence

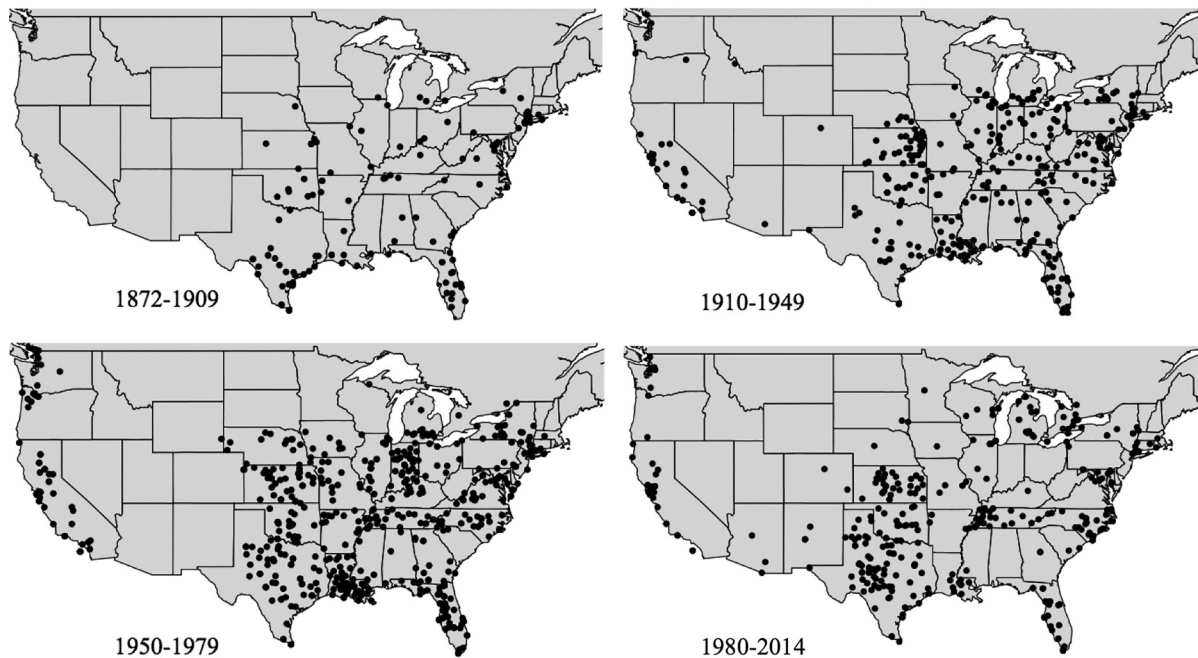
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**Fig. 1.** Map of all available VertNet records of the Virginia opossum (*Didelphis virginiana*) with location data collected in Canada and the United States per county, spanning from 1872 to 2014. A total of 2896 specimens were included in the data set. Opossum expansion on the Pacific coast was due to multiple introductions by humans (reviewed by Gardner and Sunquist 2003).



of climate change on populations, researchers have begun to examine genetic outcomes of range expansions in the context of large-scale geographic variation in climate (e.g., Wellenreuther et al. 2011).

In addition to the effects of climate change, contemporary range expansions may also be facilitated by human manipulation of the landscape and of populations. By clearing dense forests for timber and replacing the area with farmland, humans have increased available habitat and facilitated the expansion of generalists such as the coyote (*Canis latrans* Say, 1823) (Parker 1995). Where some species have capitalized on human land-use change, others have been obstructed. Some expansions have been impeded by urban sprawl, with these species relying on patches of forested areas and derelict land to expand through urban areas (Gilchrist et al. 2015). Furthermore, humans unknowingly transport organisms, both native and exotic species, to new environments. Genetic analyses have indicated that some range expansions are a combination of natural dispersal events and human-mediated introductions (Bronnenhuber et al. 2011; Moscarella 2011).

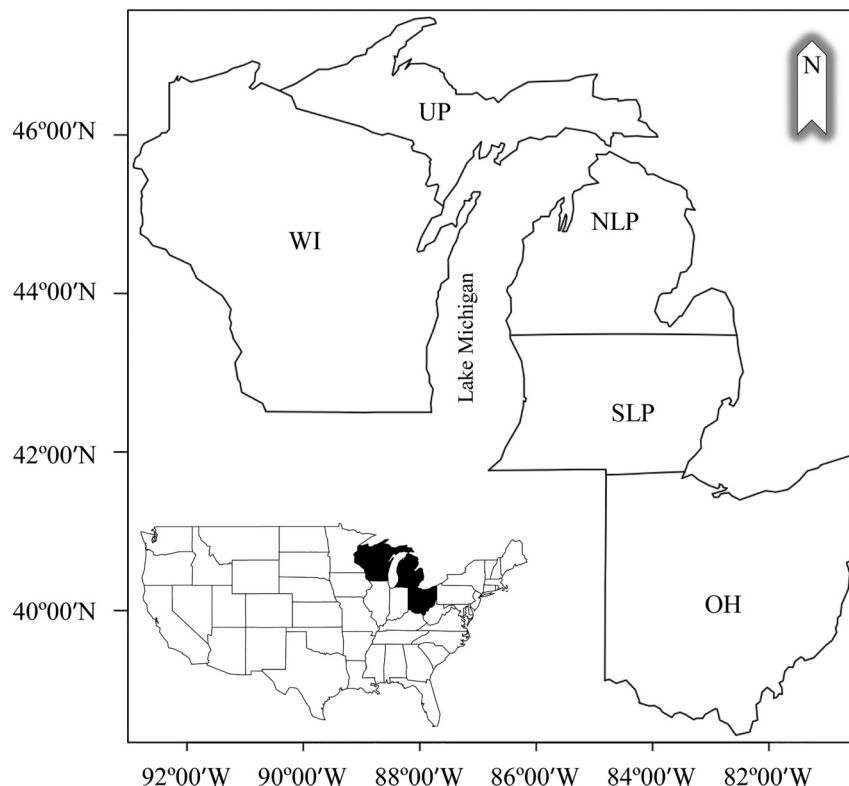
The Virginia opossum (*Didelphis virginiana* Kerr, 1792; henceforth opossum), a generalist mesopredator found from Central America to the Midwestern United States and southeastern Ontario, Canada, offers a unique opportunity to study a documented, ongoing mammalian range expansion (Fig. 1; Gardner and Sunquist 2003; Kanda 2005; Myers et al. 2009). The genus *Didelphis* L., 1758 was part of the Great American Interchange, spreading into North America from South America approximately 800 000 years ago (Graham and Lundelius 2010). Historical documentation from fur trappers and naturalists have allowed for a rough estimate of the timing of the opossum's continued expansion into the Midwestern United States. As recently as 1600, the opossum's northern range was limited to northern Ohio (reviewed by McManus 1974). Opossums were first observed in southern Wisconsin in the 1850s and, as their population grew in the 1920s, they began to spread farther north (Jackson 1961). In the adjacent Upper Peninsula (UP) of Michigan (Fig. 2), sightings of opossum in the southwestern corner became more common in the 1980s, but opossums have yet to reach the eastern half of the UP (Myers et al. 2009). It is speculated

that land clearing in the 1820s and 1830s facilitated expansion of opossum into the Lower Peninsula (LP) of Michigan (Fig. 2), but the species did not become common in southern Michigan until the 1920s (Dice 1927; Baker 1983). Opossums were first observed in the northern half of the LP in the 1940s, but were not considered well established until 1990 (Burt 1948; Myers et al. 2009).

A lack of substantial landscape barriers for opossums (Beatty et al. 2012), coupled with their nomadic behavior, illustrated by fathers and offspring discovered up to 33 km apart (Gardner and Sunquist 2003; Beasley et al. 2010), suggest that very little is preventing their continued dispersal northward, provided they can survive winters. Opossums collared with GPS units in Michigan had home ranges calculated between 10.9 and 200 ha, depending on the equation used, with no difference between sexes (Walter et al. 2013). Opossums must be able to forage at least 35 days in the winter to avoid starvation, and they do not forage when temperatures are below  $-2^{\circ}\text{C}$  or in deep snow, which hinders locomotion (Brocke 1970). Based on these observations, it has long been hypothesized that the opossum's continued expansion in Michigan was due to warming temperatures associated with climate change (Brocke 1970; Myers et al. 2009). However, a study that modeled opossum demographic data demonstrates that a winter survival rate of  $\geq 0.67$  is required to sustain opossum populations at northern latitudes (Kanda and Fuller 2004). This survival rate is higher than would be predicted based on their physiology even with winter temperatures ameliorated by climate change. Because climate change alone cannot explain why opossums have expanded so far north, Kanda (2005) proposed that urbanization has facilitated opossum survival in northern parts of their range.

The expansion of the opossum provides an opportunity to investigate its long-term movement and compare two edge populations to putative source populations that have been established for over 100 years. Peripheral populations are shaped by the length of time since initial establishment and subsequent gene flow, and are typically expected to have reduced genetic diversity compared with neighboring and central populations (Excoffier et al. 2009). Microsatellite data were used to evaluate this population genetics prediction by comparing the recently established

**Fig. 2.** To evaluate two expansion fronts, Virginia opossums (*Didelphis virginiana*) were collected from three US states: Ohio (OH), Wisconsin (WI), and Michigan. Three geographic areas were established for Virginia opossums in Michigan: the southern Lower Peninsula (SLP), northern Lower Peninsula (NLP), and the Upper Peninsula (UP).



edge populations of opossum in the northern Lower Peninsula (NLP) and UP of Michigan to more central populations in southern Michigan, Ohio, and Wisconsin, respectively (Fig. 2). The parallel expansions into UP and NLP provide an opportunity to identify correlations between genetic diversity and human and climate variables in each region to test Kanda's (2005) hypothesis that opossum winter survival is due to urbanization.

## Materials and methods

### Tissue acquisition

Tissue samples were collected from east of Lake Michigan in Ohio and LP of Michigan and west of Lake Michigan in Wisconsin and UP of Michigan (Fig. 2). Opossum tissue samples of ear, tail, lip, or hair follicles were collected from roadkill, carcasses at a wildlife rehabilitation center, and trapped specimens between 1 May and 20 November 2013 and accessioned into the University of Michigan Museum of Zoology tissue collection (MZ 11501 – MZ 11611). Except for hair, the tissue to be sampled was cleaned with an alcohol swab and collected using a sterile razor. Tissue samples were placed in DMSO tissue buffer (20% dimethyl sulfoxide saturated with NaCl) and stored at room temperature. Any hair collected was placed in a coin envelope and sealed in a plastic bag. All sampling was conducted under the direction of the University of Michigan's Committee on the Use and Care of Animals (protocol No. PRO4800).

To determine the exact location of roadkill, coordinates were taken using the Android app GPS Coordinates (Woozilli, Inc., Sunnyvale, California, USA). Ear samples were collected from opossums that had died in the wildlife rehabilitation hospital at the Ohio Wildlife Center in Columbus, Ohio, with the exact loca-

tion of each opossum provided as the roadway intersection where it had been found injured. Licensed fur trappers and nuisance control operators in both Wisconsin and UP of Michigan submitted tissue samples from opossums trapped during each state's recognized trapping season, and they provided the name of either the town or the county where they trapped opossums.

### DNA extraction and PCR

Tissue samples were processed to isolate DNA using the QIAGEN DNeasy blood and tissue kit (QIAGEN GmbH, Hilden, Germany). Concentrations of DNA ranged from 2.73 to 1115.2 ng/ $\mu$ L. Polymerase chain reaction (PCR) was conducted for 11 polymorphic microsatellite markers, optimizing the PCR protocol for each fluorescently labelled primer (Fike et al. 2009). The number of PCR cycles was increased from 30 to 40 cycles and a gradient was run for each primer pair to determine the optimal annealing temperature (Supplementary Table S1).<sup>1</sup> PCR products were submitted to the University of Michigan DNA Sequencing Core with HiDi formamide and LIZ500 ladder to be run on Applied Biosystems DNA sequencer (Applied Biosystems Inc., Foster City, California, USA) for fragment analysis. Fragment analysis results were analyzed with GeneMarker version 1.5 (Softgenetics LLC, State College, Pennsylvania, USA) using a bin-width of 0.5 to determine the length of microsatellites and establish the genotype of each opossum.

### Population genetic analyses

The number of alleles at each locus were found using FSTAT version 2.9.3.2 (Goudet 2002). The data set was tested for null alleles using Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004). Null alleles can result in an overestimation of genetic dis-

<sup>1</sup>Supplementary tables are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2017-0071>.



tance (Chapuis and Estoup 2007). Individual loci were tested for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium using Arlequin version 3.5 (Excoffier and Lischer 2010). The HWE test was run with 1 000 000 Markov chain steps and 100 000 dememorization steps. The pairwise linkage disequilibrium test was run for 10 000 permutations with 100 000 dememorization steps. After Bonferroni correction (Rice 1989), two loci (*OP16* and *OP30*) were found to violate the assumptions of HWE and linkage equilibrium. Because this can confound genetic analyses, the data from *OP16* and *OP30* were removed from the data set, and further analyses included the remaining nine microsatellite markers.

### Estimation of genetic structure

To determine the number of genetic clusters of opossums in Ohio, Wisconsin, and Michigan, a Bayesian clustering method was implemented using STRUCTURE version 2.3.4 (Pritchard et al. 2000). An admixture model with correlated allele frequencies and without location site priors was used to estimate the number of clusters ( $K$ ). Including sample location in the model could conflate the structure of opossums collected close to one another. Previous genetic structure work on opossums indicated  $K = 1$  in an area approximately 1165 km<sup>2</sup> in size (Beatty et al. 2012), so an upper limit for the broader geographic data was set at  $K = 21$ , representing approximate sample sites based on trapping and roadkill procurement. Trials were run for  $K = 1$ –21 for 20 iterations with a burn-in of 10 000 and 200 000 runs in each trial. The parameter  $\Delta K$  was used to identify the number of clusters (Evanno et al. 2005). The replicate with the highest likelihood was used to evaluate the membership coefficient ( $Q$ ) for each individual to assign samples to the clusters identified (Jakobsson and Rosenberg 2007). Genetic cluster results were assessed for substructure by analyzing individual clusters in STRUCTURE. Trials were run for 10 iterations, using the same parameter values as the previous analysis. No substructure was found.

A paired Mantel test to evaluate the relationship between geographic and genetic distance was implemented in GenAIEx version 6.5 (Peakall and Smouse 2012) to test for isolation by distance using all 85 samples. In addition, each genetic cluster identified in STRUCTURE (Pritchard et al. 2000) was individually tested for isolation by distance. Geographic distance and genetic distance were both calculated in a tri-distance matrix using GenAIEx (Peakall and Smouse 2012).

### Diversity in the expanding populations

To compare opossums at the edge of their range to their source populations, several diversity indices were calculated for each edge population and their respective putative source populations per results from STRUCTURE analysis. The edge population (UP) was compared with Wisconsin. To compare the northern edge population in the LP of Michigan, this land mass was broken into two areas using county lines to differentiate the edge population found in counties north of Gratiot County (NLP) and the southern half of Michigan's Lower Peninsula (SLP), which has been occupied by opossums since the 1800s. County borders were used because they were associated with documented historical sightings of opossums and are used by government agencies when recording census and climate data. Opossums collected in the NLP were also compared with those collected in Ohio.

Expected heterozygosity ( $H_E$ ) and observed heterozygosity ( $H_O$ ) were measured in Excel using the add-in program GenAIEx version 6.5 (Peakall and Smouse 2012), whereas the rarefied allelic richness ( $A_R$ ), which is the mean number of alleles per locus, was calculated to a standardized population ( $g$ ) of 8 using ADZE version 1.0 for each genetic cluster identified in STRUCTURE (Szpiech et al. 2008). Rarefaction accounts for the difference in sampling efforts between the different populations, which varied from 9 to 20 individuals (Kalinowski 2004). Rarefied private allele richness ( $P_R$ ), which is the number of unique alleles found in a population,

was also computed using ADZE version 1.0 (Szpiech et al. 2008) for  $g = 8$ .

### Factors affecting range expansions

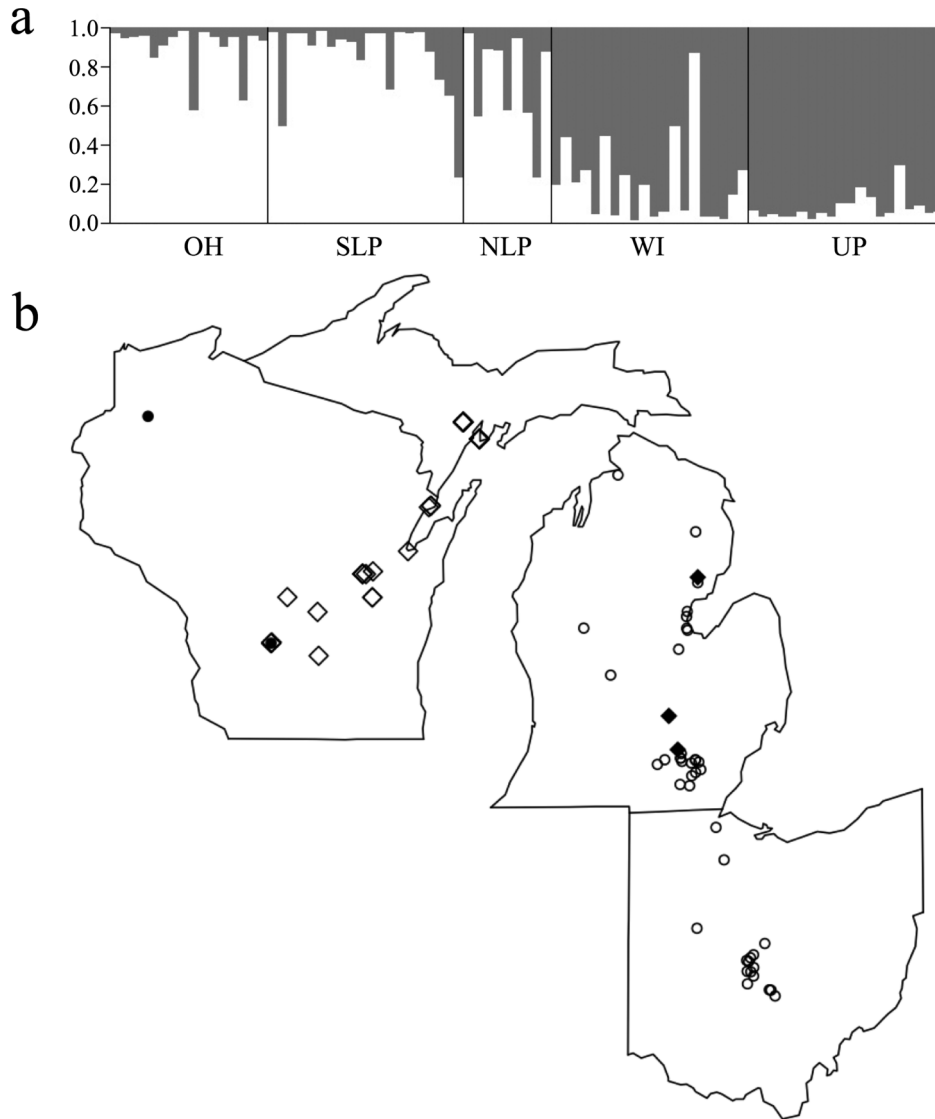
Two researchers proposed that the recent range expansion of the opossum has been facilitated by either human-provided resources (Kanda 2005) or diminished winters due to climate change (Myers et al. 2009). Habitat variables at the state level were determined to be too coarse-grained; therefore, we tested the influence of human development and climate on opossum genetic diversity at the county level. Counties with  $n \geq 3$  or multiple adjacent counties with combined  $n \geq 3$  were identified as regions to conduct a preliminary evaluation of putative influences on opossum genetic diversity. By sampling regions with 3–20 individuals, a sufficient number of regions were made available to conduct a general linear model (GLM) and at least six alleles could be used in standardizing allelic richness across regions (Szpiech et al. 2008). Although small samples sizes may not reveal the true allelic diversity, rarefaction can allow for accurate comparisons between populations. In a study comparing a diverse population to a known low-diversity population, a random sample of five individuals was sufficient to correctly reveal the low-diversity population using measures of rarefied allelic richness (Pruett and Winker 2008).

Data from each region ( $n = 11$  regions) were collected for the following anthropogenic variables: human population density, housing density (U.S. Census Bureau 2010), and density of farms (USDA NASS 2014). To calculate density measurements for regions containing multiple counties, raw census data were summed and density was calculated using the total land surface area of relevant counties (Supplementary Table S2).<sup>1</sup>

Climate data for the 11 regions were collected from 1992 to 2013, as each region has been occupied by opossums for at least 20 years (Myers et al. 2009). County data for each region were downloaded from the National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information (formerly NOAA National Climate Data Center) (available from <http://www.ncdc.noaa.gov/cdo-web/search>; accessed July 2015), averaged, and analyzed in R (R Core Team 2014) to find the annual mean maximum snow depth, 20 year extreme maximum snow depth, and annual mean number of days with at least 1 inch (2.54 cm) of snow on the ground per region. Annual mean daily minimum winter temperature (December–February) for each region was also calculated. The number of days in which the minimum or maximum temperature fell below freezing was collected as annual sums and averaged. When years had data from multiple weather stations, the values were averaged (Supplementary Table S2).<sup>1</sup>

Allelic richness and private allele richness were rarefied to a  $g = 3$  for each region using ADZE version 1.0 (Szpiech et al. 2008). There was no correlation between sample size and either  $A_R$  or  $P_R$  (Pearson coefficients =  $-0.294$  and  $0.04$ , respectively). GLMs were designed using the generalized linear model option in SPSS version 22.0 (IBM Corp. 2013) with a linear response and factorial model to examine anthropogenic variables, climate variables, and a combination. The full factorial model creates all possible interactions and main effects for variables considered within a model. Covariates were standardized using the R package “arm”, which allows for the input variables to be centered along the mean and divided by two standard deviations (Gelman 2008). An intercept-only model was created to serve as the null model. Model performances were measured using Akaike's information criterion adjusted for the number of covariates in a model ( $AIC_c$ ) and compared using  $\Delta AIC_c$  (calculated as  $AIC_{ci} - AIC_{cMIN}$ , where  $AIC_{ci}$  is the  $AIC_c$  for a given model  $i$  and  $AIC_{cMIN}$  is the lowest  $AIC_c$  across all models). Models with a  $\Delta AIC_c < 2$  are considered to have substantial support (Burnham and Anderson 2002).

**Fig. 3.** Virginia opossums (*Didelphis virginiana*) clustered according to STRUCTURE ( $K = 2$ ) based on nine microsatellite markers. (a) Bayesian clustering likelihoods for individuals. Black vertical lines separate the geographic location of individuals (Ohio (OH), southern Lower Peninsula (SLP) of Michigan, northern Lower Peninsula (NLP) of Michigan, Wisconsin (WI), Upper Peninsula (UP) of Michigan). (b) Geographic locations for individuals from both clusters. Cluster 1 (open circles;  $n = 44$ ) mostly comprises opossums from Ohio and Lower Michigan, but also includes outliers from Wisconsin (solid circles). Cluster 2 (open diamonds;  $n = 41$ ) comprises mostly opossums from Wisconsin and Michigan's UP, but also includes three outliers from Lower Michigan (solid diamonds).



One to three anthropogenic variables were used to create anthropogenic models, and all combinations of anthropogenic variables were evaluated for model performance (Supplementary Table S3).<sup>1</sup> Each climate model contained up to two covariates, but did not contain more than one variable related to temperature and snow. Models with three covariates were built using the highest performing two-covariate climate models by adding each anthropogenic variable individually. Similarly, models were built using the highest two-covariate anthropogenic models by adding each climate variable individually. Each anthropogenic variable and climate variable were paired to build combination two-covariate models.

## Results

A total of 85 opossum samples were successfully amplified for microsatellite signals. All nine loci in HWE were polymorphic, yielding between 6 and 12 alleles. The Bayesian clustering program STRUCTURE yielded the highest  $\Delta K$  when  $K = 2$ , indicating

that there are two genetic clusters represented in opossums collected from Ohio, Michigan, and Wisconsin (Fig. 3a). One cluster contained 44 individuals composed mostly of samples collected from eastern Great Lakes locations (i.e., Michigan and Ohio) with a mean membership coefficient ( $Q$ ) of 0.877, while the second cluster contained 41 individuals, most collected from western Great Lakes locations with a mean  $Q$  of 0.862 (Fig. 3b).

Across the entire sampling regime, isolation by distance was not found to be significant ( $r = 0.007$ ,  $P = 0.39$ ), and this pattern was repeated in the genetic cluster east of Lake Michigan ( $r = -0.072$ ,  $P = 0.32$ ). However, there was a faint but significant signal of isolation by distance on the western side of Lake Michigan ( $r = 0.175$ ,  $P = 0.01$ ).

## Diversity in the expanding populations

When diversity measurements in the western genetic cluster were compared between the edge, UP, and its source population (Wisconsin), UP had significantly lower  $P_R$  (Table 1). When diver-

**Table 1.** Diversity measurements of the Virginia opossum (*Didelphis virginiana*) including observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), rarefied allelic richness ( $A_R$ ), and rarefied private allelic richness ( $P_R$ )  $\pm$  standard error (SE) for the five geographic areas sampled.

Geographic area		$n$	$H_O \pm SE$	$H_E \pm SE$	$A_R \pm SE$	$P_R \pm SE$
Edge	Upper Peninsula (UP)	20	0.826 $\pm$ 0.190	0.715 $\pm$ 0.026	4.624 $\pm$ 0.330	0.783 $\pm$ 0.186
Source	Wisconsin	20	0.736 $\pm$ 0.056	0.762 $\pm$ 0.026	5.215 $\pm$ 0.360	1.374 $\pm$ 0.238
Edge	Northern Michigan (NLP)	9	0.870 $\pm$ 0.048	0.774 $\pm$ 0.018	4.719 $\pm$ 0.189	0.816 $\pm$ 0.138
Sources	Southern Michigan (SLP)	20	0.825 $\pm$ 0.03	0.774 $\pm$ 0.02	4.395 $\pm$ 0.240	0.595 $\pm$ 0.102
	Ohio	16	0.755 $\pm$ 0.05	0.752 $\pm$ 0.28	4.30 $\pm$ 0.243	0.655 $\pm$ 0.122

**Table 2.** Highest performing general linear models used to examine region-wide rarefied allelic richness ( $A_R$ ) in Virginia opossums (*Didelphis virginiana*), ranked by  $\Delta AIC_c$ .

Model	$\Delta AIC_c$	$w_i$
<b>Human density + Days w/ground snow</b>	<b>0.00</b>	<b>0.367</b>
<b>Housing density + Days w/ground snow</b>	<b>0.206</b>	<b>0.331</b>
Farm density	3.07	0.079
Farm density + Days w/ground snow	4.409	0.040
Intercept only	8.189	0.006

**Note:** Models in boldface type are considered to have substantial support (Burnham and Anderson 2002).  $\Delta AIC_c$  is the difference in a model's Akaike's information criterion adjusted for covariate size and  $w_i$  is Akaike weight. Models with  $\Delta AIC_c < 5$  were selected as the highest performing models.

**Table 3.** Highest performing general linear models used to examine region-wide rarefied private allelic richness ( $P_R$ ) in Virginia opossums (*Didelphis virginiana*), ranked by  $\Delta AIC_c$ .

Model	$\Delta AIC_c$	$w_i$
<b>Days w/ground snow</b>	<b>0.000</b>	<b>0.214</b>
<b>Freezing days</b>	<b>0.474</b>	<b>0.169</b>
<b>Housing density + Human density</b>	<b>1.708</b>	<b>0.091</b>
Intercept only	2.630	0.057

**Note:** Models in boldface type are considered to have substantial support (Burnham and Anderson 2002).  $\Delta AIC_c$  is the difference in a model's Akaike's information criterion adjusted for covariate size and  $w_i$  is Akaike weight. Models with  $\Delta AIC_c$  less than the null model (intercept only) were selected as the highest performing models.

sity measurements in the eastern genetic cluster were compared between the edge, NLP, and its two putative source populations (SLP and Ohio), there were no significant differences observed in either  $A_R$  or  $P_R$  (Table 1).

### Factors affecting range expansions

A GLM with  $A_R$  as the dependent variable had the highest support with human density and days of snow on the ground as covariates (Table 2;  $AIC_c = -21.938$ ; parameter estimates =  $-0.152$  and  $-0.234$ , respectively; SE = 0.036). The next most strongly supported model included housing density and days of snow on the ground ( $\Delta AIC_c = 0.206$ ; parameter estimates =  $-0.148$  and  $-0.228$ , respectively; SE = 0.036), followed by a model with farm density as the only covariate ( $\Delta AIC_c = 3.070$ , parameter estimate = 0.158, SE = 0.042). The highest performing model with a temperature variable included mean number of freezing days and farm density ( $\Delta AIC_c = 6.726$ ).

A GLM with  $P_R$  as the dependent variable had the highest support with days of snow on the ground as its only covariate (Table 3;  $AIC_c = -25.673$ , parameter estimate =  $-0.093$ , SE = 0.032). The next most strongly supported model included mean number of freezing days as its only covariate ( $\Delta AIC_c = 0.474$ , parameter estimate =  $-0.09$ , SE = 0.032), followed by a model with housing density and human density as the two covariates ( $\Delta AIC_c = 1.708$ ; parameter estimates =  $-2.436$  and 2.487, respectively; SE = 0.689).

### Discussion

Scientists have long sought to understand how species are distributed and the underlying forces controlling species ranges. Since the first citation of climate change as the driving force behind a contemporary range expansion (Payette 1987), it has rapidly become the most common mechanism cited for recent range expansions throughout the world (Parmesan et al. 2005; Rius et al. 2014). The opossum's range expansion was originally credited to climate change (Brocke 1970; Myers et al. 2009), but its expansion beyond its proposed climate niche in Michigan based on thermoregulation and winter foraging activity makes the Midwest populations of the opossum an optimal study system for directly evaluating other forces influencing the expansion of a mesopredator.

Bayesian clustering indicates two genetic clusters with strong geographical partitioning around Lake Michigan were represented among the opossums sampled (Fig. 3a). The large geographic range of each cluster (both greater than 100 000 km<sup>2</sup>) indicates substantial gene flow in Midwestern opossums (Fig. 3b), and coincides with previous findings that opossums across Indiana belonged to a single genetic cluster (Hennessy et al. 2015). Numerous geographic outliers suggest long-distance dispersal facilitated by humans. Although isolation by distance was tested for all samples and within each geographic cluster, only the samples west of Lake Michigan showed significant, slight isolation by distance. This suggests that distance alone cannot explain the two clusters identified on either side of Lake Michigan. The presence of two clusters of opossum, a nomadic species with negligible philopatry (reviewed by Gardner and Sunquist 2003), suggests independent colonization of the two regions on either side of Lake Michigan. A more comprehensive evaluation of the opossum's entire range using more conservative markers than microsatellites could reveal deeper historical patterns of the opossum's expansion northward since the Pleistocene (Graham and Lundelius 2010).

The diversity measurements of allelic richness and private alleles are influenced by gene flow, genetic drift, and population turnover. Larger, more stable populations are expected to have higher allelic richness and more private alleles. Newly founded populations, as well as regions with higher turnover rates, are expected to have lower allelic richness and fewer private alleles, although isolated, older populations are known to harbor unique alleles (Slatkin 1985).

When diversity measurements were compared between the edge populations and their source populations, there was a stark difference between the two edges. The UP had reduced  $P_R$  compared with Wisconsin, which is typically expected when comparing a younger, edge population to an older population. There was no significant difference in  $P_R$  when the other edge (NLP) was compared with its source populations (Table 1). In fact, this edge population had a higher number of private alleles, the opposite of what would be predicted using population genetics theory. The reduction in  $P_R$  relative to source populations in the UP but not



NLP might be explained by the  $P_R$  GLMs. The strongest model included days with snow on the ground as a negative influence. When the NLP county (Bay) is compared with the UP county (Delta), there is a 70% increase in this variable in the UP (Supplementary Table S2).<sup>1</sup> Fewer days of snow on the ground in NLP may have allowed for a larger, older population to be sustained.

Based on the lack of reduction in  $A_R$ , both expansion edges are likely to have been colonized numerous times and do not appear to be suffering from substantial bottleneck events. Alternatively, each genetic cluster as a whole may serve as edge populations when compared with more southern areas of the opossum range (e.g., Texas, Florida). A population genetics analysis across the opossum's range could reveal this information.

The GLMs evaluated in this study suggest a combination of snow variables and anthropogenic factors are shaping diversity measurements at the northwestern edge of the opossum's range. The best GLMs constructed to explain patterns of diversity (measured as  $A_R$  and  $P_R$ ) both included days of snow on the ground as a negative variable. Snow reduces an opossum's ability to forage, requiring them to use more fat storage and potentially leading to higher rates of starvation (Brocke 1970). In addition to preventing foraging, by reducing traffic and the speed of vehicles, excess snow may reduce the likelihood of roadkill, a food source used by opossums (Hobday 2010; Beatty et al. 2016).

Strongly supported models also included anthropogenic variables such as farm density, human density, and housing density (Tables 2, 3). The models indicate a positive impact on allelic richness by density of farms, suggesting that agricultural areas may support more opossums, a pattern previously observed in a mark-recapture study conducted in the Midwest (Beatty et al. 2016). This is further supported by Schoolcraft County, located immediately adjacent to Delta County sampled in the UP. As of 2013, it had not been colonized by opossums and it had approximately four times less farm density compared with any region evaluated (Supplementary Table S2).<sup>1</sup> Crops and livestock feed may be exploited in the fall by juvenile opossums, allowing them to rapidly gain mass and increase fat storage to survive winter. Beatty et al. (2014, 2016) suggested that traffic patterns on farm roads result in carrion upon which opossums would opportunistically scavenge. Research conducted in Indiana found that opossums avoided farmland in the winter (Beatty et al. 2014), surviving winters by foraging in wooded areas and along roads instead (Beatty et al. 2014, 2016). However, opossums at the northern edges of their range may be altering their foraging behavior because of increased snow levels. For example, in Massachusetts, opossums wintering in forested areas rather than suburban areas did not survive into spring (Kanda et al. 2009).

Some of the highest performing models included housing density as a negative variable. This suggests reduced gene flow and potentially higher mortality rates in urban regions. Interestingly, human density had a negative influence on allelic richness models, but a positive influence on private allele models. Regions with dense human occupation may serve as islands in a metapopulation, with reduced allelic richness and increased private alleles because of limited gene flow. Based on research conducted in New England, humans in suburban areas may be ameliorating the effects of winter by providing resources in the form of shelter, snow removal, and readily available food including trash, birdseed, and domestic pet feces (Kanda 2005). This hypothesis is consistent with the observation that opossums are absent from Schoolcraft County, which had approximately four times less human density than any Midwest region evaluated (Supplementary Table S2).<sup>1</sup> However, there may be a saturation point at which the density of urban land is too high to support large opossum populations.

The climate and anthropogenic data used to evaluate opossums collected in 2013 represent the variety of conditions in which these opossums were living, but are relatively static compared with the ongoing range expansion of the opossum. However, in

addition to the GLM results, there are historical anecdotal data to support the conclusion that land-use change has partially facilitated the opossum's expansion. For example, the opossum's initial colonization of Michigan and Wisconsin in the 1800s came prior to climate amelioration and was anecdotally credited to forest clearing (Dice 1927; Jackson 1961).

It is important to note that there may be additional important factors which have been overlooked in the models presented, especially because the null model was a competing model for  $P_R$  (Table 3). Opossums use the dens of other animals and may heavily rely on the presence of other medium-sized mammals in forested areas when seeking refuge from winter temperatures (Brocke 1970). However, models that account for the ecology of a species and their nonlinear response to climate change have been found to more accurately predict field observations, and are likely to help scientists make more realistic predictions in the face of climate change (Mordecai et al. 2013).

Based on the lack of strong support for  $A_R$  models incorporating winter temperatures, it cannot be currently concluded that temperature is a strong factor in influencing the survival of opossums at the edge of their distribution. Temperature could have once been the limiting factor (Brocke 1970), but it may now be overcome by the increased presence of agriculture in areas with dangerously low winter temperatures. Based on thermoregulation studies conducted in the 1960s, it was assumed that the opossum's northern range in Michigan was closely linked to winter temperatures (Brocke 1970; Myers et al. 2009). However, opossums have been observed beyond the temperature niche that Brocke (1970) prescribed (Kanda and Fuller 2004), and the top models in our study suggest that opossum populations are more impacted by the presence and longevity of snow than by harsh winter temperatures. The analysis of human variables and how they are related to diversity measurements suggest that agricultural lands are providing significant resources for Midwestern opossums, and with substantial gene flow across the study area, it appears that little is limiting the dispersal of opossums in this region apart from time.

Conclusions based on this study are potentially limited by sample size, i.e., 85 individuals across a large geographic area. However, based on previous population studies conducted in Indiana, in which opossums throughout the state clustered as one genetic unit, it is unlikely that substantial structure was missed with 85 opossums (Hennessy et al. 2015). Although the question of biasing samples by collecting roadkill might be raised, opossum populations have not been found to be structured by roads (Beatty et al. 2012; Hennessy et al. 2015), and roadkill opossums were found in urban, suburban, and rural areas. The analysis of putative factors influencing opossum colonization and survival is limited by using regions with low sample sizes. Although rarefaction helps mitigate discrepancies between sample sizes, the calculations of diversity are not as accurate because of the limited input of data. This limitation is especially noticeable in our  $P_R$  models, in which the intercept-only was a competitive model. These GLMs were designed as a preliminary analysis; they suggest that both climate and human land use influence opossum survival. Mark-recapture and isotope studies, as well as more widespread genetic analyses, can help further elucidate the factors ultimately driving the opossum's expansion.

In this geographic analysis of environmental factors, results suggest the opossum's range expansion has been facilitated by both climate amelioration and land clearing for agriculture. The opossum appears to have capitalized on land-use change over the past 200 years, and it is likely to continue its expansion into the northern Midwest, as evidenced by its recent appearance in North Dakota (Walsh et al. 2017). Allelic richness is used as a predictor for evolutionary potential (Kalinowski 2004); the relatively high measurements in both edge regions suggest that opossums in the Midwest have a relatively high potential for adapting to novel communities. As a scavenger, they have positive effects on ecosys-



tem services (Olson et al. 2012). However, they also carry parasites that have detrimental health effects on domestic animals including horses (Fenger et al. 1995). Because of the multiple factors influencing the opossum's successful colonization, as well as the multiple effects they will have in new areas, the opossum continues to serve as a promising system to study range expansions.

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### Supplementary Material

**Table S1.** Forward and reverse sequences and respective annealing temperatures for eleven primer pairs used to isolate and amplify polyallelic microsatellites from *Didelphis virginiana* DNA collected in Ohio, Wisconsin, and Michigan. Annealing temperatures in bold were modified from original primer protocols (Fike et al. 2009).

Primer	Sequence	Annealing temp. (°C)
OP03	F: 5'-CCTTATTGTATGTGTCCTCTTCTTTC-3' R: 5'-CTTATTTGGGATACTGGCACAC-3'	<b>66</b>
OP08	F: AAGTTGCCAAACATAGATAATAAGC R: TCACTTCTATTGTCAGATGACTTACC	<b>57</b>
OP16	F: TCTGAAAGAGGAAACAATATCAGC R: TAAGAAGCCAACCCATACCC	<b>58</b>
OP19	F: GCAATAGTCCAGGCATGAGG R: AACACCATCCTCTGGTTTGC	64
OP46	F: AGGATAGTGAGAGTTCCTTATTGG R: TTCTAAACAGTTCTGTGTATGTATTCC	64
OP31	F: GGGAGTTACTAAGGCAGAGAGTC R: TTCCTCATTCCTCCTCTATGG	65
OP14	F: GCCATAGTTGTCAGTTTCTTCC R: CTGCCTAGCTCTCAAAGTGC	<b>60</b>
OP39	F: GCCCAGCCACTTTACAGC R: CATGAATCCAAATGACTTCACC	65
OP21	F: AATTCCTGGAAGCCTCAGC	65

	R: GCCAGATGAAGCTCTAATTGC	
OP42	F: GCTTCTTTGAAAGTGAAATGTTG	<b>63</b>
	R: CATATACATTGAACAGTTGGCTATTG	
OP30	F: CTCAGACACTGAGTGGGAAGG R:	<b>63</b>
	GCCACAATGTTAGATAACAAATTCC	

Fike, J. A., J. C. Beasley, and O. E. Rhodes, Jr. 2009. Isolation of 21 polymorphic microsatellite markers for the Virginia opossum (*Didelphis virginiana*). *Molecular Ecology* 9:1200–1202.



**Table S2.** Eleven regions composed of 1-3 adjacent counties were used to examine general linear models for rarefied allelic richness ( $A_R$ ) and private allelic richness ( $P_R$ ) standardized to 3. Covariates yielding high-performing models ( $\Delta AIC_c < 2$ ) are included in the table. Schoolcraft County in the Upper Peninsula of Michigan was added in bold to provide a comparison to a proximate region in which opossums have not been detected.

Counties per region	State	$n$	$A_R \pm SE$	$P_R \pm SE$	Anthropogenic variables			Climate variables	
					Farm density	Human density	Housing density	Days w snow	Freezing days
Delaware	Ohio	3	2.600 $\pm$ 0.049	0.283 $\pm$ 0.095	1.70	393.2	149.8	14.4	110.9
Franklin	Ohio	8	2.396 $\pm$ 0.103	0.178 $\pm$ 0.034	0.729	2186.1	990.6	19.1	107.3
Bay	Michigan	4	2.545 $\pm$ 0.069	0.171 $\pm$ 0.090	1.724	242.6	108.5	33.4	137.6
Delta	Michigan	20	2.346 $\pm$ 0.040	0.103 $\pm$ 0.040	0.242	31.7	17.3	56.8	166.8
Jackson, Ingham	Michigan	3	2.325 $\pm$ 0.112	0.096 $\pm$ 0.069	0.801	348.5	150.7	50.0	141.2
Lenawee	Michigan	3	2.631 $\pm$ 0.082	0.037 $\pm$ 0.021	2.156	133.1	57.9	33.3	139.9
Washtenaw	Michigan	12	2.477 $\pm$ 0.069	0.175 $\pm$ 0.059	2.185	609.7	260.9	43.5	137.4
Adams, Marquette, Columbia	Wisconsin	3	2.331 $\pm$ 0.139	0.053 $\pm$ 0.018	0.421	49.9	28.6	64.9	157.6
Marinette	Wisconsin	3	2.413 $\pm$ 0.050	0.131 $\pm$ 0.080	0.382	29.8	21.7	56.9	161.5
Outagamie, Brown	Wisconsin	7	2.428 $\pm$ 0.033	0.151 $\pm$ 0.063	0.977	363.9	152.1	47.8	155.5
Sauk	Wisconsin	4	2.410 $\pm$ 0.084	0.088 $\pm$ 0.028	2.004	74.6	35.8	48.7	161.9
<b>Schoolcraft</b>	<b>Michigan</b>	-	<b>NA</b>	<b>NA</b>	<b>0.056</b>	<b>7.2</b>	<b>5.4</b>	<b>67.6</b>	<b>159.4</b>

**Table S3.** General linear models used to examine diversity measurements in Virginia opossums (*Didelphis virginiana*).

Null model	Intercept only
Anthropogenic models	Housing density
	Human density
	Farm density
	Housing density + Human density <sup>P</sup>
	Human density + Farm density
	Housing density + Farm density <sup>A</sup>
	Housing density + Human density+ Farm density
Climate models	Freezing days
	Days never above freezing
	Monthly minimum temp
	Days w/ground snow
	Maximum snow fall
	Average snow depth
	Freezing days + Days w/ground snow <sup>A</sup>
	Freezing days + Maximum snow fall
	Freezing days + Average snow depth
	Days never above freezing + Days w/ground snow <sup>P</sup>
	Days never above freezing + Maximum snow fall
	Days never above freezing + Average snow depth
	Monthly minimum temp + Days w/ground snow
	Monthly minimum temp + Maximum snow fall
Monthly minimum temp + Average snow depth	
Anthropogenic+	Housing density + Freezing days
	Housing density + Days never above freezing
Climate models	Housing density + Monthly minimum temp
	Housing density + Days w/ground snow
	Housing density + Maximum snow fall
	Housing density + Average snow depth
	Human density + Freezing days
	Human density + Days never above freezing
	Human density + Monthly minimum temp
	Human density + Days w/ground snow <sup>A</sup>
	Human density + Maximum snow fall
	Human density + Average snow depth
	Farm density + Freezing days
	Farm density + Days never above freezing
	Farm density + Monthly minimum temp
	Farm density + Days w/ground snow <sup>P</sup>
	Farm density + Maximum snow fall
	Farm density + Average snow depth

<sup>P</sup> = Top performing 2-covariate rarefied private allele model

<sup>A</sup> = Top performing 2-covariate rarefied allelic richness model